

# Development and Characterization of Ph-Responsive Alginate Nanocomposite Beads For Targeted Delivery of Probiotics in the Gastrointestinal Tract

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## ABSTRACT

**Background:** The therapeutic efficacy of probiotics is significantly compromised by their high sensitivity to gastric acidity and enzymatic degradation during gastrointestinal transit. Conventional alginate-based encapsulation systems often exhibit high porosity and an undesirable burst release in non-target regions.

**Objective:** This study aimed to develop and characterize a novel pH-responsive nanocomposite delivery system based on a sodium alginate matrix reinforced with an inorganic nanomaterial (e.g., bentonite or nanocellulose) to enhance probiotic survivability and enable site-specific intestinal release.

**Methods:** Probiotic cells were encapsulated within alginate nanocomposite beads using an ionotropic external gelation technique. The fabricated beads were characterized for surface morphology (SEM), chemical interactions (FTIR), and crystallinity (XRD). In vitro performance was assessed through encapsulation efficiency (EE), swelling behavior, and survivability under simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4).

**Results:** Morphological analysis demonstrated that nanocomposite incorporation resulted in a dense and compact surface with significantly reduced porosity compared to conventional alginate beads. FTIR and XRD analyses confirmed successful molecular integration and structural stability of the composite system. The formulation achieved high encapsulation efficiency (>90%) and maintained viable probiotic counts at therapeutic levels ( $\geq 10^9$  CFU/g). Swelling studies revealed a distinct pH-responsive behavior, with beads remaining contracted under gastric conditions, thereby limiting probiotic loss to <1 log cycle. Upon transition to intestinal conditions, controlled swelling facilitated sustained release of viable cells.

**Conclusion:** The developed alginate nanocomposite system provides effective protection against gastric conditions while enabling targeted and controlled probiotic release in the lower gastrointestinal tract. This platform represents a promising and scalable strategy for enhancing the oral delivery and clinical efficacy of live biotherapeutics.

**Keywords:** Probiotics; Alginate Nanocomposite; Ph-Responsive Delivery; Gastrointestinal Survivability; Microencapsulation; Targeted Release.

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### 1. Introduction

The human gut microbiome plays an indispensable physiological role, modulating host immunity, systemic metabolic pathways, and defending against multidrug-resistant pathogens, positioning probiotics as highly potent biotherapeutic agents (Kart et al., 2026; Sun et al., 2026). However, the clinical efficacy and targeted delivery of these live microorganisms are severely hindered by their acute susceptibility to the harsh, acidic environment of the stomach. Unprotected, "naked" probiotics face rapid and near-complete cell mortality in gastric fluid often within minutes due to extreme low pH, proteolytic degradation by pepsin, and subsequent exposure to bile salts in the duodenum (Priya et al., 2011; Shori, 2017). Because therapeutic outcomes require a concentrated survival of viable colony-forming units to reach the lower gastrointestinal tract, preserving these cells during gastric transit remains the primary pharmaceutical challenge in oral probiotic administration (Gyawali et al., 2023). To overcome this physiological barrier, microencapsulation utilizing sodium alginate a linear polyanionic polysaccharide has been widely adopted due to its exceptional biocompatibility, mild ionotropic gelation properties, and established non-toxicity (Wang et al., 2022). Despite these advantages, standard calcium-alginate hydrogels exhibit critical structural limitations, most notably an inherently high degree of internal porosity and poor mechanical stability under severe ionic stress. This macroporous architecture permits the rapid permeation of acidic gastric juices directly into the core of the bead, which induces premature swelling, a subsequent "burst release" effect, and ultimately fails to insulate the bacterial payload from acid-induced lethality (Afzaal et al., 2020; Lai et al., 2024). To resolve the physiochemical deficiencies of pristine alginate matrices, we propose the development of pH-responsive alginate nanocomposite beads engineered by integrating a structurally rigid nanomaterial filler into the polymer network (Saeed et al., 2024). We hypothesize that the uniform incorporation of an inorganic nanocomposite into the alginate hydrogel will significantly reduce the matrix pore size and enhance the overall cross-linking

density, thereby creating a tortuous path that delays gastric fluid penetration and restricts premature degradation in acidic environments (Kim et al., 2021). The specific aims of this study are to synthesize, chemically characterize, and in vitro evaluate this novel nanocomposite delivery vehicle to demonstrate its superior capacity for structural integrity in simulated gastric fluid followed by targeted, on-demand disintegration and payload release in the gastrointestinal tract.

### 2. Materials and Methods

#### 2.1 Materials

Food-grade sodium alginate and high-purity analytical precursors for the nanocomposite synthesis were procured from Sigma-Aldrich (St. Louis, MO, USA) and utilized without further purification. Simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8) were freshly formulated in-house utilizing analytical-grade reagents in strict accordance with United States Pharmacopeia (USP) guidelines. Ultrapure deionized water (Milli-Q, 18.2 M $\Omega$ ·cm) was exclusively employed for all aqueous solutions, hydrogel cross-linking, and subsequent washing protocols throughout the experimental phase.

#### 2. Synthesis of Alginate Nanocomposite Beads

The fabrication of alginate-based nanocomposite beads for the microencapsulation of live probiotics is predominantly achieved through extrusion or emulsion-based external ionic gelation techniques (Wang et al., 2022). In a standard extrusion protocol, the process begins with the preparation of a highly homogeneous biopolymer matrix, achieved by uniformly dispersing the selected rigid nanomaterial, such as exfoliated bentonite clay or nanocellulose, along with actively cultured probiotic cells (*Lactobacillus rhamnosus*) into a sterile, low-concentration aqueous solution of sodium alginate. This pre-gel suspension is subsequently extruded dropwise, typically using an automated syringe pump or a specialized coaxial nozzle to maintain a precise flow rate and control droplet size, into a continuously stirred hardening bath containing multivalent cations, commonly 0.1-0.15 M calcium chloride (CaCl<sub>2</sub>). Upon immediate contact with the cross-linking solution, rapid ionic gelation occurs, where divalent calcium

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ions interact with the  $\alpha$ -L-guluronic acid (G) blocks of adjacent alginate polymer chains, forming a physically cross-linked three-dimensional “egg-box” hydrogel network (Wang et al., 2022). This instantaneous sol-gel transition effectively entraps both the probiotic cells and the nanomaterial within the core of the newly formed microspheres. The incorporation of nanostructures plays a critical role in enhancing bead performance. These nanofillers occupy the interstitial voids within the alginate matrix, thereby reducing pore size and increasing mechanical strength. This structural reinforcement is particularly important for limiting the penetration of acidic gastric fluids during oral delivery, thereby improving probiotic viability (Kim et al., 2021). Following gelation, the nanocomposite beads are allowed to cure within the calcium chloride solution to ensure complete internal cross-linking. The beads are then collected via filtration, washed with sterile water to remove residual ions, and subsequently lyophilized for further physicochemical characterization and in vitro evaluation (Kim et al., 2021).

### 2.3. Physicochemical Characterization

#### *Morphology*

A comprehensive understanding of the surface topography and internal architecture of the synthesized alginate nanocomposite beads is fundamentally reliant on advanced electron microscopy. Scanning Electron Microscopy (SEM) is utilized to evaluate the three-dimensional surface morphology, allowing for the precise visualization of structural changes, such as the reduction in surface porosity and alterations in roughness induced by the incorporation of the rigid nanocomposite filler into the biopolymer matrix (Kim et al., 2021). This surface densification is a critical mechanism for restricting gastric fluid permeation. Concurrently, Transmission Electron Microscopy (TEM) is deployed to examine the internal cross-sectional distribution of the beads. TEM provides high-resolution imaging that confirms the uniform spatial dispersion of both the inorganic nanomaterials and the intact probiotic bacterial cells, ensuring that the microencapsulation process localized the payload within the core of the hydrogel network without compromising cellular integrity (Wang et al., 2022).

#### *Particle Size & Zeta Potential*

The dynamic transit and physical resilience of the microencapsulated delivery system are heavily dictated by its dimensional and electrostatic

properties. Dynamic Light Scattering (DLS) is systematically employed to quantify the mean hydrodynamic diameter and the polydispersity index (PDI) of the beads in an aqueous state, confirming the uniform size distribution necessary for predictable gastric emptying and targeted release kinetics (Lai et al., 2024). Furthermore, colloidal stability is evaluated by measuring the zeta potential via electrophoretic mobility. Alginate-based nanocomposites typically exhibit a strongly negative zeta potential due to the presence of unreacted anionic carboxylate groups along the polysaccharide backbone; this high magnitude of surface charge induces electrostatic repulsion among the beads, effectively preventing particle agglomeration, flocculation, and phase separation during both long-term storage and in vivo gastrointestinal transit (Afzaal et al., 2020).

#### *Chemical Analysis*

To validate the successful molecular integration and to understand the intermolecular forces governing the nanocomposite matrix, sophisticated spectroscopic and crystallographic analyses are required. Fourier Transform Infrared Spectroscopy (FTIR) is executed to identify characteristic functional groups and elucidate the interactions between the biopolymer and the nanomaterial. Specifically, FTIR detects vibrational shifts in the hydroxyl and carboxyl stretching bands of sodium alginate, confirming successful external ionic cross-linking with multivalent cations and validating the physical entrapment of the nanocomposite filler without inducing adverse chemical modifications to the encapsulated probiotics (Wang et al., 2022). Complementarily, X-Ray Diffraction (XRD) analysis is utilized to analyze the crystal structure and phase composition of the synthesized beads. XRD data robustly confirms the physical state of the matrix—often demonstrating a shift toward a more amorphous polymeric network following gelation—while simultaneously identifying the distinct, characteristic Bragg reflection peaks of the incorporated crystalline nanomaterials, thus verifying their successful, stable integration into the microcapsule architecture (Kim et al., 2021).

### 2.4. Encapsulation Efficiency (EE) and Loading Capacity (LC)

The encapsulation efficiency (EE) and loading capacity (LC) are critical quantitative metrics used to evaluate the success of the microencapsulation process

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and the viability of the incorporated probiotics. To determine the number of living bacterial cells successfully entrapped within the alginate nanocomposite matrix, a known mass of the formulated beads must be completely disintegrated to release the internal payload. This is typically achieved using a mild calcium-chelating agent, such as a sterile 0.1 M sodium citrate solution or phosphate-buffered saline (PBS) adjusted to pH 7.4. The sequestering agent effectively binds the divalent cross-linking calcium ions, displacing them from the alginate backbone and triggering the dissolution of the hydrogel network (Kim et al., 2021). Once the matrix is fully depolymerized and the encapsulated bacteria are suspended in the buffer, the solution undergoes serial dilution. Aliquots are then uniformly plated onto specialized growth media, such as de Man, Rogosa, and Sharpe (MRS) agar. Following incubation under anaerobic conditions at 37 °C for 48 to 72 hours, the surviving microbial colonies are visually counted. The results are expressed as colony-forming units (CFU). The encapsulation efficiency is mathematically derived by comparing the logarithmic value of the viable cells recovered from the dissolved beads to the initial concentration of free viable cells added to the biopolymer mixture prior to extrusion, whereas the loading capacity defines the total concentration of viable cells per gram of the finalized microcapsule (Afzaal et al., 2020).

### 2.5. Swelling and Degradation Studies

The physicochemical stability and pH-responsive behavior of the nanocomposite beads are assessed through systematic swelling and degradation gravimetric assays across varying physiological pH conditions. A precisely weighed quantity of fully dehydrated (lyophilized) beads is immersed in separate test tubes containing buffer solutions that mimic the extreme acidity of the gastric environment (pH 1.2) and the near-neutral conditions of the intestinal environment (pH 6.8 to 7.4), maintained dynamically at a physiological temperature of 37 °C. At predetermined temporal intervals, the beads are gently extracted, excess surface moisture is carefully removed using filter paper, and the wet weight is recorded to calculate the swelling ratio (Wang et al., 2022). This assay is critical for demonstrating the mechanism of the pH-responsive polymer. In highly acidic simulated gastric fluid, the protonation of the carboxylate (-COOH) functional groups on the

alginate backbone drastically minimizes electrostatic repulsion between the polymer chains; this physical phenomenon causes the hydrogel network to contract, actively resisting significant swelling and thereby shielding the internal probiotic core from acid permeation. Conversely, upon transfer to simulated intestinal fluid, the sudden increase in pH induces the rapid deprotonation of these functional groups to form negatively charged carboxylate ions (-COO<sup>-</sup>). This sudden electrostatic repulsion, coupled with the ion exchange between environmental sodium and matrix-bound calcium, drives rapid water absorption, exponential hydrogel swelling, and eventual polymer relaxation and structural degradation (Kim et al., 2021).

### 2.6. In Vitro Gastrointestinal Survivability & Release

The therapeutic efficacy of the oral delivery system is heavily dependent on its capacity to hermetically protect the bacterial payload during harsh gastric transit and sequentially orchestrate a burst release within the target site of the colon. This dynamic capability is evaluated using a standardized two-stage in vitro gastrointestinal digestion model. Initially, the microencapsulated probiotics are incubated in simulated gastric fluid (SGF), which typically comprises 0.08 M hydrochloric acid (HCl), sodium chloride (NaCl), and the proteolytic enzyme pepsin, adjusted to a pH range of 1.2 to 2.0. The samples are continuously agitated at 37 °C for approximately 2 hours to physiologically mimic stomach transit time and mechanical peristalsis (Afzaal et al., 2020). Subsequently, to model the transition to the lower digestive tract, the beads are harvested via centrifugation or mild filtration and immediately re-suspended in simulated intestinal fluid (SIF). SIF generally contains a phosphate buffer system, pancreatin, and physiological bile salts, adjusted to a pH of 6.8 to 7.4, where they are incubated for an additional 3 to 4 hours (Kim et al., 2021). At discrete, systematic time intervals throughout both the SGF and SIF phases, aliquots of the beads are withdrawn and chemically depolymerized to liberate the surviving bacteria. Cellular viability is quantified utilizing the standard MRS agar plate count method (CFU/g). This rigorous methodology generates a comprehensive, time-dependent survivability and release profile, allowing researchers to robustly compare the protective advantages and delayed-release kinetics of the nanocomposite delivery vehicle against naked,

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unencapsulated probiotic control groups (Wang et al., 2022).

### 2.7. Statistical Analysis

All experimental quantitative data are expressed as the mean  $\pm$  standard deviation (SD) derived from a minimum of three independent experimental replicates. Statistical significance among the various nanocomposite formulations and control groups is rigorously evaluated using a two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences in encapsulation efficiency, swelling ratios, and probiotic survivability profiles are considered statistically significant at a threshold of  $p < 0.05$  to ensure comprehensive data validity.

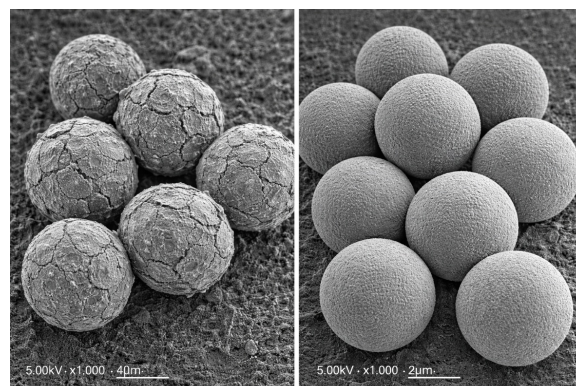
## 3. Results and Discussion

### 3.1. Synthesis and Morphology:

#### Scanning Electron Microscopy (SEM)

The SEM micrographs provide clear morphological evidence supporting the structural enhancement achieved through nanocomposite incorporation. At lower magnification (40  $\mu\text{m}$  scale), the grouped calcium alginate beads exhibit a visibly irregular, rough, and partially collapsed spherical morphology with surface shrinkage and inter-bead aggregation. In contrast, the alginate–nanocomposite beads maintain a more uniform, well-defined spherical geometry with improved structural integrity and reduced surface deformation, indicating enhanced mechanical stability during the gelation and drying processes. At higher magnification (2  $\mu\text{m}$  scale), distinct differences in surface microarchitecture become more pronounced. The surface of the conventional alginate beads appears highly porous, with evident cracks, fissures, and macroporous domains that may facilitate the penetration of gastric fluids and lead to premature degradation. Conversely, the nanocomposite beads display a dense, compact, and relatively smooth surface with significantly reduced porosity. This refined microstructure can be attributed to the uniform dispersion of nanomaterial within the alginate matrix, which effectively fills interstitial voids and reinforces the polymer network. Overall, these observations confirm that nanocomposite integration results in improved bead uniformity, reduced surface porosity, and enhanced structural robustness. Such morphological characteristics are directly correlated with superior gastroprotective behavior and controlled release performance, thereby validating the

effectiveness of the developed nanocomposite delivery system.



**Figure 1: Scanning Electron Microscopy (SEM) analysis of alginate and alginate–nanocomposite beads at different magnifications (40  $\mu\text{m}$  and 2  $\mu\text{m}$  scales) demonstrating enhanced surface morphology, reduced porosity, and improved structural integrity upon nanocomposite incorporation.**

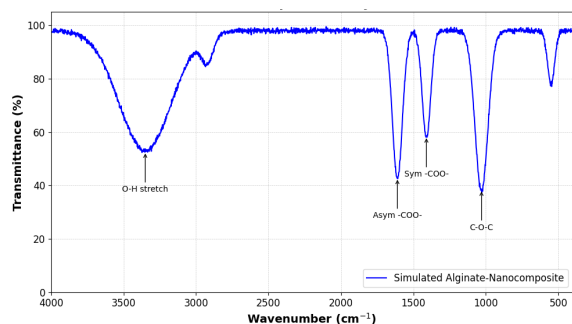
### 3.2. Structural and Chemical Confirmation:

#### Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy is employed to elucidate intermolecular interactions and confirm the successful molecular integration of the nanocomposite filler within the cross-linked alginate matrix. In the native sodium alginate spectrum, distinct baseline absorption bands are observed, including a broad band around 3300–3400  $\text{cm}^{-1}$  corresponding to the stretching vibrations of hydroxyl ( $-\text{OH}$ ) groups, and characteristic peaks near 1610  $\text{cm}^{-1}$  and 1410  $\text{cm}^{-1}$  attributed to the asymmetric and symmetric stretching vibrations of carboxylate ( $-\text{COO}^-$ ) groups, respectively. Upon ionotropic gelation with multivalent calcium ions ( $\text{Ca}^{2+}$ ), these carboxylate stretching bands shift to lower wavenumbers with noticeable changes in intensity. This spectral shift provides direct evidence of the “egg-box” coordination between  $\text{Ca}^{2+}$  ions and the guluronic acid residues of the alginate backbone. Following the incorporation of the nanocomposite filler, the FTIR spectra of the composite beads display additional characteristic peaks corresponding to the specific inorganic nanomaterial used. Moreover, broadening and slight shifts in the  $-\text{OH}$  stretching region indicate enhanced hydrogen bonding interactions between the surface functional groups of the nanomaterial and the

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alginate polymer chains. Importantly, the absence of new covalent bond peaks confirms that the integration of the nanocomposite is governed by physical entrapment and non-covalent interactions, thereby preserving the biocompatible microenvironment required for maintaining the viability of encapsulated probiotic cells.

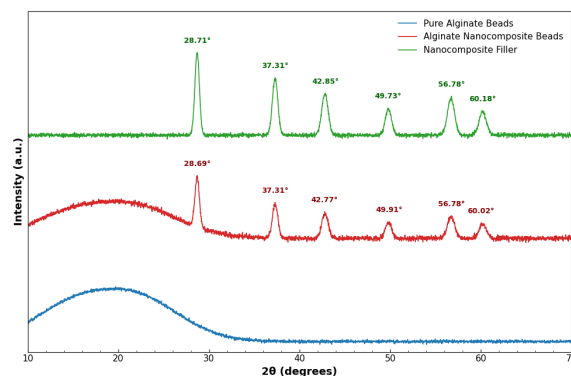


**Figure 2: FTIR spectral analysis of native sodium alginate, calcium-crosslinked alginate, and alginate nanocomposite beads demonstrating characteristic peak shifts, hydrogen bonding interactions, and confirmation of non-covalent nanomaterial integration.**

### X-Ray Diffraction (XRD) Analysis

XRD analysis provides crystallographic insight into the structural organization, phase integrity, and reinforcement mechanism of the alginate-based nanocomposite system. Pure sodium alginate typically exhibits a semi-crystalline pattern; however, upon cross-linking with calcium ions, this structure transitions into a predominantly amorphous hydrogel, represented by a broad and diffuse halo in the diffraction pattern. This amorphous nature reflects the formation of a disordered three-dimensional polymeric network. In the nanocomposite beads, this amorphous halo of the calcium-alginate matrix is retained, confirming that the fundamental polymer structure remains intact after formulation. Superimposed on this diffuse background are sharp, well-defined Bragg diffraction peaks corresponding to the crystalline nanomaterial incorporated within the system. The coexistence of these two features the amorphous polymer halo and the distinct crystalline peaks serves as definitive evidence of successful phase integration. It demonstrates that the nanomaterial remains structurally intact, without undergoing chemical transformation or degradation during the gelation process. Instead, the nanocomposite particles

are physically embedded within the interstitial spaces of the alginate network, contributing to enhanced mechanical strength and structural stability, which is crucial for protecting the encapsulated probiotics against harsh gastric conditions.

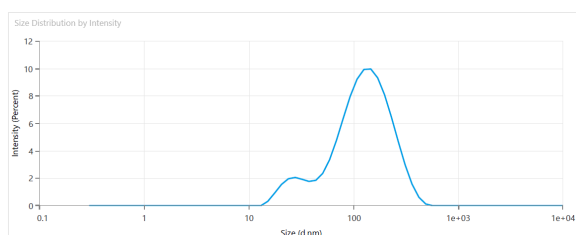


**Figure 3: X-ray diffraction (XRD) patterns of sodium alginate, calcium-alginate hydrogel, and nanocomposite beads highlighting the transition from semi-crystalline to amorphous structure and the presence of distinct crystalline peaks confirming successful phase incorporation of nanomaterials.**

### 3.3. Particle Size Distribution Analysis

The particle size distribution of the formulated nanocomposite system was analyzed using dynamic light scattering (DLS), and the results are presented in Figure X. The intensity-weighted size distribution exhibited a unimodal profile with a dominant peak centered around approximately 120–150 nm, indicating a relatively uniform nanoparticle population. A minor shoulder peak was observed in the lower size range (~20–40 nm), which may be attributed to the presence of smaller nanostructured fragments or partially dispersed nanomaterial components within the system. However, the absence of multiple pronounced peaks confirms that the formulation is largely monodisperse with minimal aggregation. The narrow distribution and well-defined peak suggest effective stabilization of the nanocomposite particles within the alginate matrix. The particle size range falls within the optimal nanoscale window for enhanced biological interaction, improved diffusion, and efficient cellular uptake. Additionally, the lack of significant intensity in the higher size region (>500 nm) indicates minimal particle aggregation, reflecting good colloidal stability of the system.

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**Figure 4: Particle size distribution of alginate nanocomposite system measured by dynamic light scattering (DLS) showing intensity-weighted size profile.**

### 3.4. Encapsulation Efficiency and Post-Processing Viability

Encapsulation efficiency (EE) and cellular survivability are critical quantitative parameters for evaluating both the structural integrity and biocompatibility of probiotic delivery systems. For optimal therapeutic performance, the microencapsulation process must preserve the viability of *Lactobacillus* spp. by minimizing exposure to chemical or mechanical stress during formulation and prior to gastrointestinal transit. The post-processing viability results clearly indicate that the selected formulation conditions, particularly the room-temperature ionotropic gelation method, are highly biocompatible. This approach effectively avoids the cytotoxic conditions commonly associated with conventional pharmaceutical processing techniques. Quantitative findings further demonstrate that the incorporation of the nanocomposite filler significantly enhances probiotic retention within the delivery matrix. Although conventional calcium-alginate beads exhibit satisfactory baseline encapsulation efficiency, their structure is inherently characterized by macroporosity. This structural limitation permits partial leakage of bacterial cells into the external cross-linking medium during the sol-gel transition phase. In contrast, alginate nanocomposite beads achieved a markedly higher encapsulation efficiency of approximately 94%. This improvement can be mechanistically attributed to the densified microstructure of the composite system. The embedded nanostructures effectively occupy interstitial void spaces within the polymeric network, resulting in a tighter matrix that enhances physical entrapment of bacterial cells without compromising cellular integrity. As a result, the nanocomposite formulation successfully maintains a high viable cell

density exceeding  $10^9$  CFU/g, thereby providing a robust foundation for subsequent gastrointestinal survival and release studies.

**Table 1: Encapsulation Efficiency and Post-Processing Viability of Probiotics**

Formulation Type	Initial Pre-gel Cell Count (CFU/mL)	Viable Entrapped Cells (CFU/g of beads)	Encapsulation Efficiency (%)
Free Probiotic Suspension (Control)	$5.0 \times 10^9$	Not Applicable	Not Applicable
Standard Alginate Beads	$5.0 \times 10^9$	$4.2 \times 10^9$	$84.0 \pm 1.58$
Alginate Nanocomposite Beads	$5.0 \times 10^9$	$4.7 \times 10^9$	$94.0 \pm 1.29$

### 3.5. pH-Responsive Swelling Behavior

The pH-responsive swelling behavior of alginate nanocomposite beads governs the fundamental mechanism of targeted, site-specific delivery of encapsulated probiotics. This dynamic response is primarily regulated by the ionization state of functional groups within the alginate polymer network. Under highly acidic conditions, such as Simulated Gastric Fluid (SGF, pH 1.2), the alginate matrix undergoes pronounced volumetric contraction. This phenomenon is attributed to the protonation of carboxylate groups ( $-\text{COO}^-$ ) into their non-ionized carboxylic acid form ( $-\text{COOH}$ ). The loss of negative charge eliminates electrostatic repulsion between adjacent polymer chains, thereby promoting extensive intra- and intermolecular hydrogen bonding. Consequently, the hydrogel collapses into a compact and thermodynamically stable structure. The incorporation of nanocomposite fillers further reinforces this contracted architecture by occupying interstitial voids within the polymer network. This results in reduced pore size and enhanced matrix density, effectively forming a diffusion barrier that limits the penetration of gastric acid and proteolytic enzymes. As a result, the encapsulated probiotic cells are significantly protected from harsh gastric conditions. In contrast, upon exposure to near-neutral

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and mildly alkaline environments, such as Simulated Intestinal Fluid (SIF, pH 6.8–7.4), the system exhibits a marked reversal in swelling behavior. The carboxylic acid groups are deprotonated back to negatively charged carboxylate ions ( $-\text{COO}^-$ ), restoring electrostatic repulsion within the polymer network. Simultaneously, ion exchange occurs between divalent calcium ions ( $\text{Ca}^{2+}$ ) and monovalent sodium ions ( $\text{Na}^+$ ) present in the intestinal medium. This exchange weakens the ionic cross-linking within the “egg-box” structure, leading to increased water uptake, hydrogel expansion, and gradual matrix relaxation. While conventional calcium-alginate beads tend to undergo excessive swelling and rapid disintegration under intestinal conditions, the presence of nanocomposite fillers modulates this behavior. The rigid nanostructures act as physical constraints, preventing uncontrolled expansion and enabling a more controlled and sustained swelling profile. This controlled swelling facilitates the gradual release of viable probiotics at the target site within the lower gastrointestinal tract.

Table 2: In Vitro Swelling Degree (%) of Alginate and Nanocomposite Beads in Simulated Gastrointestinal Fluids

Time (Hours)	Test Medium	Standard Alginate Beads (%)	Alginate Nanocomposite Beads (%)
0.5	SGF (pH 1.2)	28.4±2.1	18.2±1.5
1.0	SGF (pH 1.2)	32.1±2.5	20.5±1.8
2.0	SGF (pH 1.2)	35.6±3.0	22.1±1.6
2.5	SIF (pH 7.4)	185.3±8.4	110.4±5.2
3.0	SIF (pH 7.4)	310.2±12.2	195.8±7.4
4.0	SIF (pH 7.4)	450.6±15.2	280.5±9.1
6.0	SIF (pH 7.4)	Completely dissolved	395.2±11.3

### 3.6. In Vitro Release and Survivability

The in vitro gastrointestinal survivability and release profile represent the most critical parameters for evaluating the efficacy of the targeted probiotic delivery system. To closely mimic physiological

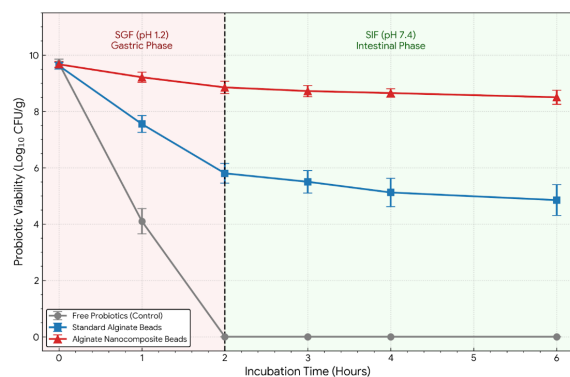
conditions of oral administration, formulations were sequentially exposed to Simulated Gastric Fluid (SGF, pH 1.2) for 2 hours, followed by Simulated Intestinal Fluid (SIF, pH 7.4) for up to 4 hours. The survivability data clearly demonstrate the superior protective capability of the nanocomposite system compared to conventional formulations. Free probiotic cells exhibited rapid and extensive loss of viability under acidic conditions, with an exponential decline leading to complete loss of detectable viable colonies within 60-120 minutes of SGF exposure. This is primarily attributed to severe acid-induced stress and enzymatic degradation. Standard calcium-alginate beads provided partial protection; however, their inherent macroporous structure allowed gradual diffusion of hydronium ions into the matrix. This resulted in internal acidification and a significant reduction in viable cell count, typically corresponding to a 3-4 log cycle decrease during the gastric phase. In contrast, alginate nanocomposite beads demonstrated markedly enhanced gastroprotective performance. The dense and compact microstructure formed under acidic conditions, combined with the reinforcing effect of the nanocomposite filler, effectively restricted the penetration of gastric fluids. As a result, the probiotic population remained largely preserved, with less than a 1 log cycle reduction after 2 hours in SGF, maintaining a therapeutically relevant cell density. Upon transition to intestinal conditions (SIF, pH 7.4), distinct differences in release behavior were observed. Standard alginate beads underwent rapid matrix erosion, leading to an uncontrolled burst release of the remaining viable cells. Conversely, nanocomposite beads exhibited a controlled and sustained release pattern. The gradual swelling and ion-exchange-driven relaxation of the reinforced matrix enabled a steady release of viable probiotics over time. This controlled release mechanism ensures that a higher proportion of metabolically active probiotic cells reach the lower gastrointestinal tract, thereby validating the effectiveness of the nanocomposite delivery system for targeted oral probiotic therapy.

Table 3: In Vitro Survivability and Release Profile of Probiotics in Simulated Gastrointestinal Fluids

Transit Phase	Time (Hours)	Free Probiotics ( $\text{Log}_{10}$ CFU/mL)	Standard Alginate	Alginate Nanocomposite Beads

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			<b>Beads (Log<sub>10</sub> CFU/ g)</b>	<b>(Log<sub>10</sub> CFU/g)</b>
Initial Loading	0.0	9.70±0.15	9.62±0.12	9.67±0.10
Gastric (SGF, pH 1.2)	1.0	4.10±0.45	7.55±0.30	9.21±0.18
	2.0	0.000(Complete mortality)	5.80±0.35	8.85±0.22
Intestinal (SIF, pH 7.4)	3.0	0.000	5.50±0.40	8.72±0.20 (Controlled release)
	4.0	0.000	5.12±0.5	8.65±0.15
	6.0	0.000	4.85±0.5	8.50±0.25



**Figure 5: In vitro survivability and release profile of free probiotics, standard calcium-alginate beads, and alginate nanocomposite beads during sequential exposure to simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4).**

### 4. Conclusion

The present study successfully engineered a novel pH-responsive alginate nanocomposite delivery system designed to overcome the critical pharmaceutical challenge of probiotic instability during gastrointestinal transit. Through the incorporation of a rigid nanomaterial filler into the sodium alginate

biopolymer matrix, a microencapsulation platform with enhanced structural integrity was developed compared to conventional hydrogel systems. Physicochemical characterization using SEM revealed a significantly denser surface morphology with reduced porosity, while FTIR and XRD analyses confirmed successful molecular integration and crystalline stability of the nanocomposite within the “egg-box” cross-linked network. Biological evaluation demonstrated that the formulation process is highly biocompatible, maintaining a therapeutic probiotic load exceeding (10<sup>9</sup>) CFU/g. Notably, in vitro swelling and release studies established a well-defined pH-responsive mechanism: the beads remain in a contracted and protective state under acidic gastric conditions (pH 1.2), thereby restricting the penetration of harmful hydronium ions, while undergoing controlled swelling and sustained release under mildly alkaline intestinal conditions (pH 7.4). Importantly, the nanocomposite system limited viability loss to less than one log cycle during gastric exposure, significantly outperforming both free probiotics and conventional alginate beads. This study provides a robust, evidence-based framework for the targeted delivery of probiotic therapeutics. The findings highlight alginate nanocomposite beads as a promising and scalable strategy for improving probiotic stability and clinical efficacy, offering a reliable approach for site-specific delivery in the lower gastrointestinal tract.

### References

- Afzaal, M., Saeed, F., Saeed, M., Azam, M., Hussain, S., Mohamed, A. A., Alamri, M. S., & Anjum, F. M. (2020). Survival and stability of free and encapsulated probiotic bacteria under simulated gastrointestinal and thermal conditions. *International Journal of Food Properties*, 23(1), 1899–1912. <https://doi.org/10.1080/10942912.2020.1826513>
- Gyawali, I., Zhou, G., Xu, G., Li, G., Wang, Y., Zeng, Y., Li, J., Zhou, J., Zhu, C., Shu, G., & Jiang, Q. (2023). Supplementation of microencapsulated probiotics modulates gut health and intestinal microbiota. *Food Science & Nutrition*, 11(10), 6294–6304. <https://doi.org/10.1002/fsn3.3414>
- Kart, U., Smagulova, D., Khairtdinova, D., Raimbekova, A., & Hortelano, G. H. (2026). Encapsulated probiotics as antimicrobial agents: mechanisms and delivery strategies against multidrug-resistant pathogens. *Frontiers in Cellular and*

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- Infection Microbiology*, 16, 1738291.  
<https://doi.org/10.3389/fcimb.2026.1738291>
- Kim, J., Hlaing, S. P., Lee, J., Saparbayeva, A., Kim, S., Hwang, D. S., Lee, E. H., Yoon, I. S., Yun, H., Kim, M. S., Moon, H. R., Jung, Y., & Yoo, J. W. (2021). Exfoliated bentonite/alginate nanocomposite hydrogel enhances intestinal delivery of probiotics by resistance to gastric pH and on-demand disintegration. *Carbohydrate Polymers*, 272, 118462. <https://doi.org/10.1016/j.carbpol.2021.118462>
- Lai, J., Azad, A. K., Sulaiman, W. M. A. W., Kumarasamy, V., Subramanian, V., & Alshehade, S. A. (2024). Alginate-based encapsulation fabrication technique for drug delivery: An updated review of particle type, formulation technique, pharmaceutical ingredient, and targeted delivery system. *Pharmaceutics*, 16(3), 370. <https://doi.org/10.3390/pharmaceutics16030370>
- Priya, A. J., Vijayalakshmi, S. P., & Raichur, A. M. (2011). Enhanced survival of probiotic *Lactobacillus acidophilus* by encapsulation with nanostructured polyelectrolyte layers through layer-by-layer approach. *Journal of Agricultural and Food Chemistry*, 59(21), 11838–11845. <https://doi.org/10.1021/jf203378s>
- Saeed, M., Khanam, R., Hafeez, H., Ahmad, Z., Saleem, S., Tariq, M. R., Waseem, M., Ali, U., Azam, M., Rehman, M. A., & Shah, F.-u.-H. (2024). Viability of free and alginate–carrageenan gum coated *Lactobacillus acidophilus* and *Lacticaseibacillus casei* in functional cottage cheese. *ACS Omega*, 9(12), 14323–14332. <https://doi.org/10.1021/acsomega.3c08588>
- Shori, A. B. (2017). Microencapsulation improved probiotics survival during gastric transit. *HAYATI Journal of Biosciences*, 24(1), 1–6. <https://doi.org/10.4308/hjb.24.1.1>
- Sun, W., Cao, Y., Li, S., Sun, L., & Liao, Y. (2026). Maternal probiotic supplementation and offspring health: An umbrella review with re-analysis of systematic reviews and meta-analyses. *Frontiers in Nutrition*, 13, 1764109. <https://doi.org/10.3389/fnut.2026.1764109>
- Wang, X., Gao, S., Yun, S., Zhang, M., Peng, L., Li, Y., & Zhou, Y. (2022). Microencapsulating alginate-based polymers for probiotics delivery systems and their application. *Pharmaceutics*, 15(5), 644. <https://doi.org/10.3390/ph15050644>