REVIEW ARTICLE

# Colon targeting and microencapsulation of probiotics: A comprehensive review

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## Abstract

Maintaining physiological balance and disease prevention depends much on the human microbiota, especially the gut microbiota. Disruptions in microbial populations are linked to various health disorders, including inflammatory, metabolic, and autoimmune diseases. Probiotics, which are live microorganisms that provide health benefits when administered in adequate amounts, have emerged as promising biotherapeutics. However, their viability through the harsh gastrointestinal tract remains a significant concern. This review highlights the importance of colon-targeted delivery systems and explores various strategies, such as pH-, time-, microbe-triggered, and combination-targeting approaches, for effective probiotic release at the colonic site. Furthermore, it provides an in-depth discussion of microencapsulation technologies, including coacervation, extrusion, emulsification, spray drying, and advanced techniques such as spray-freeze drying, electrospinning, and fluid bed coating. Emphasis is placed on the mechanisms, materials, advantages, and limitations of each method. A comprehensive understanding of colon physiology and encapsulation science is essential for developing effective formulations that ensure probiotic viability and therapeutic efficacy. Future research should focus on optimizing these systems to enhance site-specific delivery, shelf stability, and large-scale applicability.

**Key words:** Colon-targeted drug delivery, gut health, microbiome, microencapsulation, microspheres, polymer coating, prebiotics, probiotics

## INTRODUCTION

The microbiome plays an essential role in maintaining the physiological balance of overall health in the human body. It accompanies us from birth and co-evolves with human development. Several microorganisms colonise the human body, equal to the number of somatic cells, which host more than 1000 bacterial species.[1] Microbiota inhabit the skin, oral cavity, nasal cavity, and various other locations throughout the body; among them, gut microbiota is the most prominent one.[2,3] Human diseases are intricately linked to the diverse microbiota throughout our bodies. With approximately three trillion bacteria residing within us, these microorganisms regulate physiological processes and influence disease susceptibility. Their impact highlights the complex interplay between our health and the unseen life within.[4]

There is a strong relationship between microbiota imbalance and the occurrence of many diseases. Many studies have shown that colitis,[5] diabetes,[6] and other conditions are linked to this imbalance. Beyond the gut, respiratory tract microbiota imbalances are associated with chronic obstructive pulmonary disease,[7] while oral microbiota changes relate to periodontitis.[8] In addition, alterations in pulmonary microbiota can influence autoimmune diseases of the central nervous system,[9] highlighting the significant impact of microbiota on our health. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[10] Certain clinical studies have discovered that probiotics can play a preventative and supportive role in treating various diseases. For example, Lactobacillus casei Zhang has been shown to help minimise kidney damage and slow the progression of renal decline,[11]

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while *Lactobacillus plantarum* P9 can enhance conditions related to chronic constipation[12] and *Bacillus coagulans* strain LBSC has demonstrated effectiveness in reducing symptoms of irritable bowel syndrome, including bloating, abdominal discomfort, constipation, diarrhea, nausea, vomiting, and stomach rumbling.[13]

The human diet, affluent in non-digestible carbohydrates, called prebiotics, significantly influences the composition of our gut microbiota. The beneficial microbes thrive by extracting energy from the breakdown of indigestible bonds, thereby playing a vital role in our overall health and well-being. Prebiotics are defined as "a substrate that is selectively utilized by host microorganisms, conferring a health benefit."[14] Combining probiotics with these prebiotics is more effective than using probiotics alone.

To guarantee the targeted action of probiotics, it is essential to implement various targeting techniques. Furthermore, probiotics must be effectively encapsulated in suitable systems to safeguard them against experimental conditions and the harsh acidic gastric environment. Microencapsulation is a highly effective approach that has garnered significant attention and research interest. It involves enclosing cells within a protective membrane to prevent injury or loss of the cells, ensuring that microorganisms are released appropriately in the gut.[15] Enzyme-responsive and/or pH-sensitive polymers are used to create colon-targeted microspheres. These polymers inhibit the release of medication in the stomach and small intestine, allowing the drug to be released under colonic conditions through pH-sensitive or enzymatic degradation. In addition, these microspheres are coated with various sensitive polymeric materials to slow down medication release in the upper gastrointestinal tract (GIT).[16]

Given the importance of probiotics for human health and wellness, this article aims to review various techniques for targeting and microencapsulating probiotics and their various components and advantages.

## COLON PHYSIOLOGY

Grasping the physiological characteristics of the colon is vital for specifically tailoring medications. The colon consists of four main sections: The ascending, transverse, descending, and rectum, measuring approximately 1.6 m in a typical GIT of 6 m [Figure 1]. It plays a key role in the absorption of water, minerals, and vitamins, the digestion of polysaccharides, and the regulation of intestinal immunity. The colonic microbiota, which is highly diverse, represents over 70% of the body's microbes and has a unique composition akin to a fingerprint. Several bacterial groups can colonise the colon more easily because of its prolonged transit time (more than 30 h), despite being shorter than the small intestine. Understanding its distinct characteristics is vital for a thorough understanding of the digestive system.[17,18]

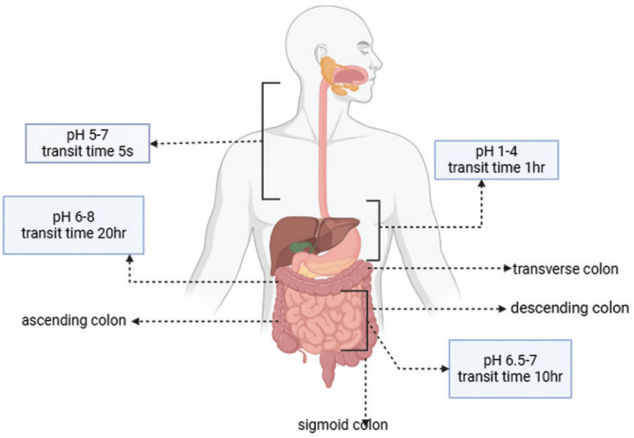


**Figure 1:** Schematic representation of the colon

### Transit Time and Physiology

The movement within the colon plays a crucial role in medication absorption, as it enhances the duration of contact between the medication and the mucosal surface. Understanding this process is vital for optimizing drug delivery and efficacy, especially for treatments aimed at colon-related conditions.[19] Factors such as food intake, particularly fatty foods, peristalsis, and the form of the medication can affect how quickly drugs transit through the colon. In addition, the rate at which medication leaves the stomach is influenced by factors such as age, gender, and health conditions, such as diabetes, which can also impact the time it takes for medication to reach the colon.[20,21]

### Colonic Contents

Mucus is a fluid that is sticky and formed by specific cells of the colon that coat the surface in a protective layer. This barrier keeps drugs from coming into direct contact with the colon's interior, which could change how the drug enters our bodies. The colon is also home to a wide variety of helpful bacteria. These bacteria can alter some pharmaceuticals and produce new compounds that may impact how well our bodies absorb medications.[22,23]

The proximal colon, including the cecum, is the main site for fermentation, whereas the distal colon primarily focuses on absorbing fluids and electrolytes. In a healthy individual, the primary bacterial phyla in the colon are Firmicutes (which comprise 90% of the microbiota), Bacteroidetes, Proteobacteria, Actinobacteria, and Verrucomicrobia. Notable genera include butyrate-producing bacteria such as *Ruminococcus* and *Clostridium* from Firmicutes, as well as Bacteroides from Bacteroidetes. The outer mucus layer of the colon plays a critical role in supporting microbial colonization. Colonic bacteria generate short-chain fatty acids (SCFAs), such as acetic, propionic, and butyric acids, which are vital for human health. Butyric acid is particularly important as an energy source for colon cells and has

anticarcinogenic effects, while propionic acid also aids in energy metabolism.[18,24]

### Colonic pH

For the solubility and dissolution of drugs, the pH variation along the GIT plays a crucial role. The stomach maintains an acidic pH, whereas the small intestine experiences a gradual increase in pH due to the secretion of bicarbonate-rich fluids from the pancreas. In the colon, the pH is generally acidic in the proximal region but shifts toward a nearly neutral pH in the distal colon.[25]

### Water Absorption and Drug Concentration

Water absorption and drug concentration are closely linked to the colon's functionality and the consistency of fecal matter. One of the primary roles of the colon is to absorb water, electrolytes, and vitamins from the luminal contents. This absorption creates a concentration effect, leading to increased medication levels in the colon.[26]

### Drug Absorption in the Colon

Drugs that are low in molecular weight, lipophilic, and relatively stable in the intestinal environment have a higher likelihood of being absorbed in the colon.[19] While the absorption capacity of the large intestine may not match that of the small intestine, a noteworthy amount of absorption is taken care of by the process of passive diffusion across the colonic epithelium.[27,28]

### Effect of Diseases on the Colonic Function

The influence of various disease states on colonic drug absorption is significant and multifaceted. Chronic inflammatory bowel diseases (CIBDs), such as Crohn's disease and ulcerative colitis (UC), are marked by persistent inflammation and damaging structural changes in the colonic mucosa. These conditions not only disrupt the digestive system's natural balance but also significantly hinder the absorption of medications. In addition, spastic bowel syndrome (SBS) requires specialized medication management, as it can greatly affect patients' well-being by altering colonic motility.[29,30]

### Colon-targeted Delivery of Probiotics

By knowing the physiology of the colon and the characteristics of probiotics, it is crucial to design the appropriate colon-targeted delivery devices.

## PROBIOTICS DELIVERY TO THE COLON

In the early 20$^{th}$ century, the pioneering scientist Elie Metchnikoff unveiled a remarkable discovery: A beneficial gut microbe that not only restores balance to gut health but also holds the key to longevity. This groundbreaking finding later earned the name "probiotic," forever changing our understanding of health and wellness.[31] Probiotics are essential health-promoting microorganisms that play a crucial role as next-generation bio-therapeutics in the field of gut microbiomics. Key bacterial species, including *Lactobacillus*, *Lactococcus*, Bacillus, *Streptococcus*, *Bifidobacterium*, *Pediococcus*, and *Propionibacterium*, are recognized as effective probiotics [Figure 2]. In addition, certain yeasts, such as *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, and *Saccharomyces boulardii*, along with fungi such as *Aspergillus niger* and *Aspergillus oryzae*, are also classified as probiotics and contribute significantly to gut health.[32] Probiotics play an important role in gut health, but their benefits extend beyond this to include brain function, boosting immunity, reducing cholesterol, and promoting metabolic homeostasis through their biological mechanisms in the body. Probiotics can produce SCFAs, vitamins, enzymes, organic acids, and antimicrobial peptides.[33]

Probiotics are primarily consumed orally and are available in several effective forms, including functional foods, dietary supplements, and medicinal products. These options allow individuals to easily integrate probiotics into their daily lives to harness their significant health benefits.[34] The stability of probiotics is a critical concern for ensuring effective delivery to the colon after oral ingestion. To optimize their efficacy, their viability must be maintained during the gastrointestinal (GI) transit. Probiotics require defence against a variety of stresses during digestion, storage, and processing. Different strains exhibit variations in their functional properties, stability, and efficacy. Strategies to enhance probiotic stability include stress adaptations through pre-treatment, mutagenesis, selective pressure treatments, and genetic modifications utilizing omics technologies.[35] However, some of these methods may impact the inherent qualities of probiotics, and genetically modified strains often face resistance in food applications. Encapsulation has proven to be the most effective method for protecting probiotics, ensuring their stability while maintaining the native properties of the strains.[36,37]

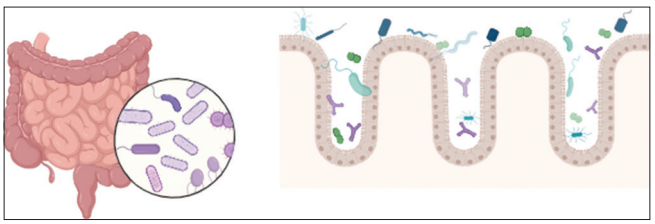

**Figure 2:** Colonic microbiome

## COLON TARGETING TECHNIQUES

Targeted drug delivery to the colon is highly beneficial for the localized treatment of several bowel conditions, including UC, Crohn's disease, amebiasis, colon cancer, localized therapy for colonic disorders, and the systemic administration of protein and peptide medications.[38,39] The colon-specific drug delivery system needs to ensure that the active agent is well-protected during its journey to the colon. This means that its release and absorption should not happen in the stomach or the small intestine, and the bioactive agent must remain intact without degradation at these earlier stages. The release and action of probiotics should be specifically targeted to occur only once the system reaches the colon.[40,41]

### pH-dependent Targeting

Drug formulations that dissolve at higher pH levels are more suited for site-specific delivery since the pH in the colon (apart from the ascending colon) and the final segment of the ileum is higher than in other areas of the GIT.[42] Even though several disorders can cause pH changes, investigation into pH-dependent systems that target the colon is still progressing. Enteric-coated microspheres represent a straightforward approach to developing pH-controlled systems for colonic drug delivery.[43] These coatings inhibit drug release in the upper GIT and contain polymers such as Eudragit, which dissolve between pH values of 6.0 and 7.0. Adjusting these polymers' proportions allows controlled drug release within this pH range. However, Eudragit S alone may not be sufficient for effective colon-specific delivery.[40] Clinical trials have indicated that these systems can struggle to release medications when the ascending colon's intestinal pH drops to 6.0. To address this issue, a combination of Eudragit polymers can ensure drug release even when the GI pH falls below 6.8.[44]

### Time-dependent Targeting

Time-dependent targeting allows the bioactive agent to become available in the colon immediately after a specific duration with time-released formulations.[45] This approach relies on the small bowel's transit time, estimated to be around 3–4 h. Gastric emptying times can vary among individuals and can be influenced by food intake.[46] In addition, conditions affecting the colon, such as short bowel syndrome (SBS) and UC, may alter large bowel transit times.[47] Scientists have created colon-specific medication administration by combining a timed-release technique with pH-sensitive polymers. This technique encases a drug core between three polymer layers, an inner water-loving layer surrounded by two pH-responsive layers.[48] Dissolution tests indicate that this design allows for sustained drug release due to pH protection and hydrogel formation.

### Microbe-triggered Targeting

A microbially controlled delivery system effectively targets the colon by utilizing specific enzymes from gut bacteria, making it ideal for colon-specific drug delivery despite pH fluctuations in the GIT. The gut microbiota, particularly in the colon, generates enzymes such as glycosidases and azoreductases that aid in the breakdown of various substances. Natural polysaccharides such as dextran and guar gum have been explored as potential options for colon-targeted drug delivery systems.[49] However, these polysaccharides generally fail to form robust films and can swell within the GIT, leading to premature drug release. Milojevic *et al.* investigated amylose, a key starch component, as a viable film-forming polymer. While amylose can gel and create films, it is prone to degradation by colonic enzymes and resistant to pancreatic alpha-amylase. Its water swelling can be controlled by incorporating materials such as acrylates or ethyl cellulose.[50] According to Lorenzo-Lamosa *et al.*,[51] pH-responsive polymers should be used to cover polysaccharide frameworks. The researchers developed chitosan microspheres that encapsulated medication and coated them with Eudragit polymers using a two-step process. They employed spray drying to keep the drug within the microspheres and then carried out solvent evaporation for microencapsulation. In laboratory tests, these Eudragit-coated microspheres did not release the drug at stomach pH. However, upon the Eudragit coating dissolving at the appropriate pH, the chitosan microspheres expanded and released the drug, which should increase in the colon. For treating amoebiasis, a multiparticulate system of chitosan microspheres coated with Eudragit is proposed for delivering metronidazole.[52] The drug is intended to release when the Eudragit degrades in the small intestine, followed by further release in the colon. Chitosan was crosslinked with glutaraldehyde to avert early drug loss, and the effectiveness of these systems was validated by observing heightened drug release in the presence of rat cecal contents, indicating the chitosan matrix's ability to degrade in the colon.

### Combination Targeting

Combination strategies for drug delivery involve the integration of multiple targeting methodologies to enhance precision in delivering pharmaceuticals to the colon. Combining time-dependent release mechanisms, microbial enzyme-dependent processes, and pH sensitivity, this synergistic method maximizes drug release kinetics and targeting efficacy. Such an integrated framework is designed to improve the overall effectiveness of therapeutic interventions.

#### *Combination of pH and time-dependent targeting*

pH-dependent and time-dependent targeting involves creating microspheres with coatings that protect the drug in the stomach and small intestine while allowing its release

in the colon. These microspheres remain unchanged in the upper GIT and release medication once they reach the colon. To develop a reliable colonic drug delivery system, Gupta *et al.*[53] aimed to utilize the small intestine's transit time of 3–4 h and elevated pH levels of about 7–8 in the lower part. They coated pellets with Eudragit RL and RS on the inside and added an outer layer of Eudragit FS 30D, which dissolves at pH levels above 6.5. This method is easy to scale and effectively targets the colon by preventing drug release below pH 6.5. Another study coated mesalamine pellets with or without inulin, using Eudragit RS and a mixture of Eudragit L and S.[54] These pellets demonstrated better therapeutic results in an animal UC model than in the pH-only triggered Pentasa formulation. There was no significant difference between the pellets with and without inulin, suggesting that both mechanisms provided added benefits.

### Combination of pH and microbe-triggered targeting

#### Phloral

Phloral® devices have established themselves as leaders in the commercial market by employing dual-triggered technology for colonic medication administration [Figure 3]. This system utilizes a single coat composed of Eudragit S and water-resistant starch.[55] The distinct triggering mechanisms of these components allow them to complement and compensate for each other's activity if one fails. Eudragit S protects the formulation's integrity during transit through the stomach and small intestine while functioning as a structuring agent for the resistant starch, controlling its expansion.[56] The resistant starch, in turn, provides an alternative pathway for drug release if the Eudragit S pH activation threshold is not met. The Phloral® approach has consistently demonstrated significant effectiveness in managing CIBD regardless of patient dietary conditions, and it has also shown efficacy in treating Clostridium difficile infections.[57,58]

#### Opticore

OPTICORE™, short for optimized colonic release, is an innovative hybrid technique designed to swiftly release medications at the ileocolonic site, where fluid concentrations surpass those in the mid and distal sections of the large intestine.[59] This cutting-edge technology utilizes a dual-layer coating system that consists of an inner layer of Eudragit® S combined with a buffering agent and an outer Phloral® layer [Figure 4].[60] For acidic drugs, an additional Hydroxypropyl methylcellulose (HPMC) coat may be added to avoid interaction with the alkaline base layer. The OPTICORE™ system improves ileocolonic drug release by promoting the degradation of the Phloral® coating, which forms pores that allow luminal fluid to penetrate. This mechanism enhances the pH, ionization strength, and buffering capacity of the remaining Phloral® coating, resulting in quicker drug dissolution and release. This system is particularly effective in administering mesalamine to inflamed areas within the colon, supporting the treatment of CIBD.[61] Asacol™, a medication using the OPTICORE™ method, has completed Phase III clinical trials in Europe and can provide up to 1.6 g of mesalamine, the highest dose approved for oral administration.[62,63] This method improves patient compliance by decreasing the frequency of dosing and has also been investigated for managing Clostridioides difficile infections through the ileocolonic delivery of metronidazole benzoate.[64]

### Combination microbe and time-triggered targeting

Although extensive research has been conducted on microbiota and time-dependent triggers for colonic drug delivery, their combined application is still uncommon.[65] An effective strategy utilizes pectin combined with HPMC as a coating, taking advantage of pectin's susceptibility to microbial degradation and HPMC's ability to swell in the GIT.[66] A pilot study demonstrated that this coating successfully delivered medications through the ascending and transverse colon in six healthy male subjects. Furthermore, studies on injection-molded capsule shells made from HPMC and high-amylose starch indicated that the colonic environment promotes improved drug release via microbial metabolism. HPMC facilitates controlled release during the swelling process. Adjusting the proportions of polymers, the thickness of the shell, and the design could enhance drug delivery, creating opportunities for innovative formulations for targeted release in specific colonic areas.[67]

### Combination of pH, microbiota, and time-dependent system

A novel medication delivery method that targets the colon and uses several activation pathways was presented by Moutaharrik *et al.*[68] Either HPMC or hydroxypropyl cellulose (HPC), a time-sensitive swellable cellulose component, is encapsulated in a mixture of a pH-sensitive polymer (Eudragit® S) and a microbiota-responsive polysaccharide (Amylo

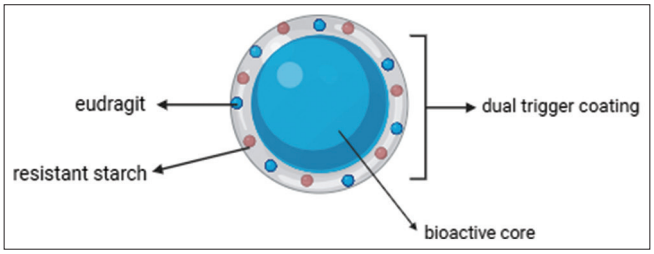


**Figure 3:** Phloral coating

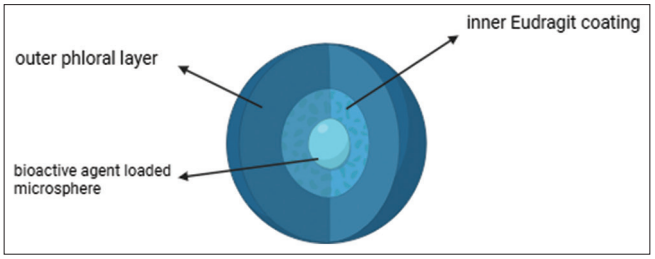


**Figure 4:** Opticore coating

N460) in this dual-layer coating system. This combination effectively exhibited colon targeting in both *in vitro* and *ex vivo* investigations, marking the first successful application of a triple targeting technique. The encouraging first findings suggest a great deal of room for improvement in *in vivo* models.

## MICROENCAPSULATION OF PROBIOTICS

The targeting techniques allow the probiotics or other bioactive materials to specifically reach the colon. For the viable agents to have their positive effects, they must reach the colon. The "therapeutic minimum" level of probiotics for positive effects is between $10^6$ and $10^8$ cfu/mL/day, which is an adequate quantity of live agents.[69] However, the high survival of probiotics in dietary supplements is linked to several issues. Several parameters, such as pH, acetic and lactic acid concentrations, hydrogen peroxide, dissolved oxygen content, titratable acidity, and species and strains of associative fermented food product microbes, may be to blame for this. Microencapsulation has been regarded as an effective and innovative method for enhancing the viability of probiotics in food products and the digestive tract since probiotic microorganisms that are intended to colonize the host gut to provide health benefits must survive.[70-72] "Microencapsulation is defined as a process in which tiny droplets of active substances are coated with a minuscule capsule. This capsule is often called a shell capsule, external phase, carrier material, matrix, or membrane [Figure 5]."[73] The therapeutic compounds are protected by the encapsulation from a variety of physical–chemical stressors, including heat, humidity, pH, and harmful chemicals.[74,75]

### Coacervation Technique

The most popular and promising encapsulation method for probiotics is coacervation. It operates on the theory that a liquid phase rich in polymers can arise when it is in equilibrium with another liquid phase. This is a colloidal phenomenon where the two distinct colloid-rich phases can show up in either a highly dispersed state or a lowly dispersed state. Numerous writers have studied how probiotics are affected by the coacervation process. Typically, dryness causes coacervated probiotics to remain in their dispersed form, which raises the expense of packaging, storing, shipping, and distributing them.[76] To dehydrate coacervated probiotics, several methods, such as spray drying, sprouting bed drying, freeze drying, etc., are used because liquid probiotics are less stable than dry ones. When electrostatic attraction is present in water, both positive and negative polymers undergo associative phase separation. These two colloids with opposing charges interacted to produce complicated coacervation. This method works well for creating and constructing the nano/microcapsulation system to improve probiotics' miscibility, storage capacity, controlled release, and deliverability.[76-78] Coacervation-produced microcapsules have exceptional controlled-release attributes (release rate retardation), which are altered by variations in temperature, pH, and ionic strength. These metastable structures are also very adaptable and dynamic, allowing them to react to changes in their surroundings.[79]

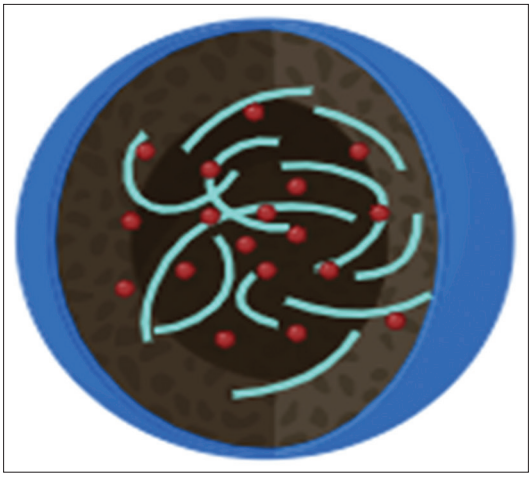

**Figure 5:** Microsphere loaded with bioactive agent

### Extrusion Technique

The extrusion technique is the most widely used approach due to its ease of use, affordability, and mild formulation conditions that guarantee excellent cell viability. This method, also known as prilling, is easy, inexpensive, and has a good retention rate of encapsulated probiotics due to its mild settings.[80,81] A hydrocolloid solution is made, microorganisms are added, and the cell suspension is extruded through a syringe needle. A hardening solution is dripped into the droplets.[82] Probiotics are mostly protected from extreme external pressures during storage by alginate biopolymer solutions and carrageenan. The hydrocolloid solution is combined with the probiotic microbes to create a suspension, which is then extruded through a syringe [Figure 6]. The hydrocolloid solution and probiotic suspension are then allowed to settle in the hardening solutions. Divalent cations, such as calcium or magnesium, make up the hardening solution. The size and shape of the extruded beads depend on several variables, including the distance between the needle and the hardening solution, the diameter of the needle, the hardening solution's surface tension, and the kind of cations utilized in the solution. This technique creates beads that range in diameter from 2 to 5 mm. Extrusion technology's impact on *Lactobacillus acidophilus* was investigated.[83] discovered that probiotic *L. acidophilus* living in milk (stored at 4°C for 50 days) or acidic water (pH 2) was more likely to survive when co-extrusion technology was used. Furthermore, mixes including alginate and other natural polysaccharides are commonly used to form particles via the extrusion method, which can extend the lifetime of encapsulated cells in storage and simulated gastric and intestinal environments.[84] The extrusion technique has a few drawbacks as well, including large bead

sizes for many uses, restricted application, decreased storage survival, and decreased encapsulating efficiency.

### Emulsification Technique

Emulsification is a widely used technique in food processing to encapsulate vitamins, minerals, enzymes, and beneficial microbes. This is because the steps involved in creating the encapsulated particles by emulsification are simple, as are the selection of ingredients needed for formulation and preparation conditions. Emulsification creates microcapsules with smaller particles than extrusion, yet it is a kinder and easier procedure [Figures 7 and 8]. A large-scale production is better suited for the emulsification process, and the addition of microcapsules somewhat alters the flavour of certain products.[85,86] The impact of cellulose acetate phthalate emulsion, calcium alginate, ę-Carrageenan-locust bean gum, alginate-starch, sodium alginate, and alginate-chitosan on the probiotics simulated in GI conditions has been investigated by many authors.[87-90] This method works with a variety of hydrocolloids and probiotic microbes, and it is highly adaptable and simple to scale up.[91] However, it does have several drawbacks, such as decreased cell stability during storage, restricted application in wet form, and high cost because of the requirement for surfactant, emulsifier, and vegetable oils.

### Cocrystallization

A prevalent method in food systems for encapsulating the active substances within the crystals is cocrystallization. Supersaturated sucrose syrup is kept at high temperatures (over 120°C) to prevent crystallization, and an active component is added as part of the encapsulation process. To support the product agglomeration leading to nucleation (crystal formation), the concentrated sucrose solution is mixed with a desired amount of active material and forcefully stirred to create the sucrose-active material mixture. Crystals vary in size between 3 and 30 µm. The active substance in this procedure is a secondary ingredient, whereas sucrose is the principal ingredient. When the solution is allowed to cool gradually, both the primary and secondary components achieve simultaneous crystallization. Orange peel oil, essential oils, honey, brown sugars, yerba mate extract, probiotics, and other ingredients were all encapsulated using this technique.[92-95] After being screened to a consistent size, the encapsulated goods are dried to the appropriate moisture content. Encapsulation using the cocrystallization approach is cost-effective and enhances separation, wettability, and solubility. In addition, it provides versatility in the management and preservation of different active substances.[96]

### Compression Coating

This method is commonly utilized for compound generation in the pharmaceutical and nutraceutical industries. It has

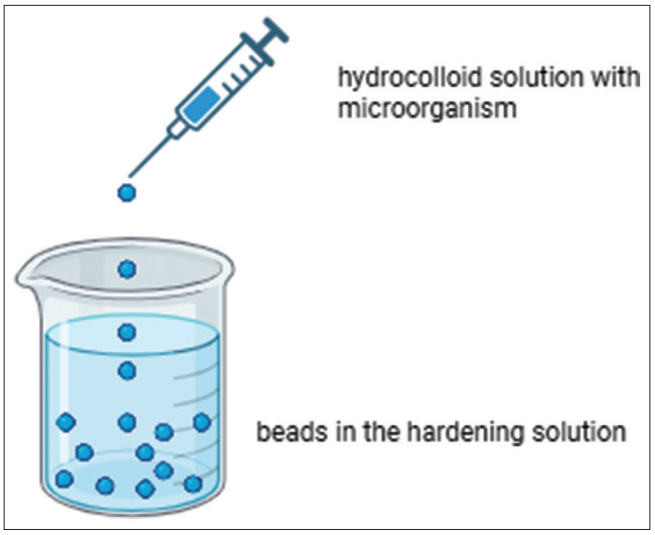


**Figure 6:** Extrusion technique

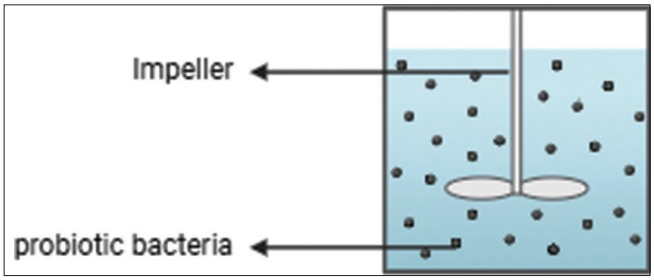


**Figure 7:** Encapsulation by emulsification

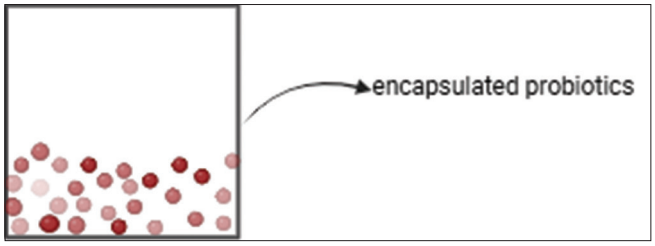


**Figure 8:** Encapsulated probiotics

the compressing coating substance, pellets or core tablets, and dried bacterial powder. The dried bacterial powder is compressed into a core tablet, which is subsequently squeezed again to encapsulate it with an appropriate coating material. Compression coating compounds include guar gum, sodium alginate, pectin, HPC, and sureteric HPMC phthalate [Figure 9].[97] When these coating materials are exposed to a dissolving fluid, a viscous gel layer forms around the core material. The stiffness of HPC was superior to that of other covering materials. However, sodium alginate, which is commonly used in the food industry, is a perfect coating material. However, it has limitations, such as low compressibility because of its elastic nature. Numerous authors (Riaz and Masud)[98] have investigated how different probiotic microorganisms are affected by compression coating. By employing compression coating with gel-forming polymers, the stability of lyophilized probiotic bacteria during storage was greatly enhanced. The compression parameters and polymer coating have a major

effect on probiotic viability. Since cell viability declines linearly with increasing pressure, the compression pressure should be kept below 90 MPa to prevent a lowering of cell viability. At 60 MPa, bacteria that have been compression-coated exhibit ten times the stability of free cell powders after 30 days at 25°C.[99] However, the tablet core cannot withstand organic solvents or water; therefore, this technique produces larger products and necessitates specialized apparatus.[100]

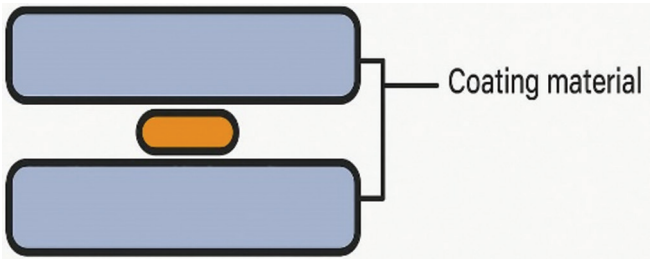


**Figure 9:** Encapsulation by compression coating

## Spray Drying Technique

This special drying method allows for the continuous generation of probiotic powder particles. The room for drying is then sprayed with liquid stock culture.[101] For encapsulating lactic acid and other probiotic cultures together with various carrier materials, some authors have proposed that the spray drying procedure is one of the sanitary, affordable, energy-efficient, and long-term preservation approaches.[102] Probiotics remain alive even after drying at higher temperatures (150–200°C) because of their rapid drying and continual synthesis [Figure 10]. The challenges of handling and preserving liquid stock culture are resolved by this procedure.[103,104]

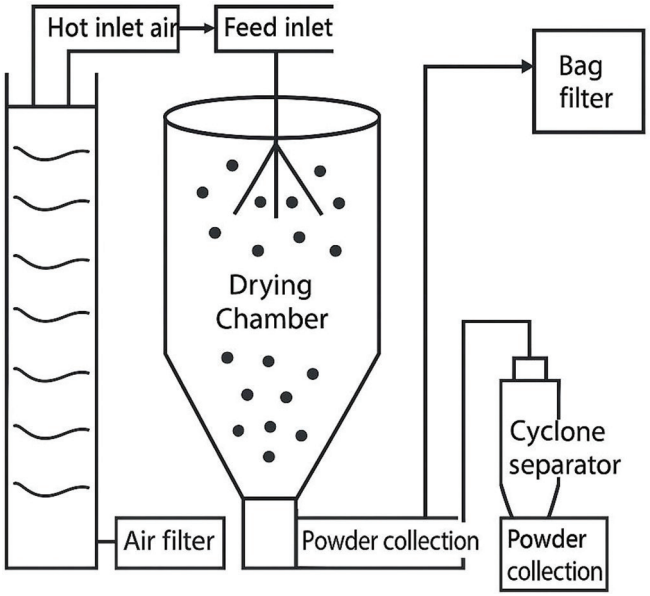


**Figure 10:** Encapsulation by spray drying

Studies have shown that bacteria may be spray-dried without losing their cell viability and activity.[105] In addition to probiotic bacteria, spray drying is frequently used to encapsulate a variety of heat-sensitive compounds, such as volatile nutrients, colours, pigments, flavours, aromas, and lipids.[106] In the same way that many authors have reported on the use of spray drying in the encapsulation of various heat-sensitive chemicals in food,[107-112] authors have reported on the encapsulation of fragrance compounds.[113] There had been a publication on oil preservation against oxidation by Fuchs *et al.*[114] A 2007 study by Nunes and Mercadante found that lycopene encapsulation is possible and can achieve a yield of 94% to 96%.[115] The size and shape of the encapsulated microcapsules, which range from 0.2 to 5000 μm, are determined by the sample's composition and preparation technique.[116] They have certain drawbacks, such as the probiotic cells' loss of viability when dried at high temperatures within a spray drying chamber.

## Spray-Freeze Drying Technique

Combining spray drying and freeze drying, spray–frozen drying is a revolutionary and distinctive drying method [Figure 11].[117] This method is frequently used to encapsulate active ingredients, dry high-value foods, and produce pharmaceutical items. The three-step process of atomising, freezing, and drying the feed solution is known as spray-freeze drying. After coming into touch with a cold fluid, the atomized droplets solidify and undergo sublimation at low temperatures and pressures.[118] Only by the atomization of the feed solution, which provides a more uniform temperature field for heat transmission during the freezing stage, is it possible for fine ice crystals to form and nucleate

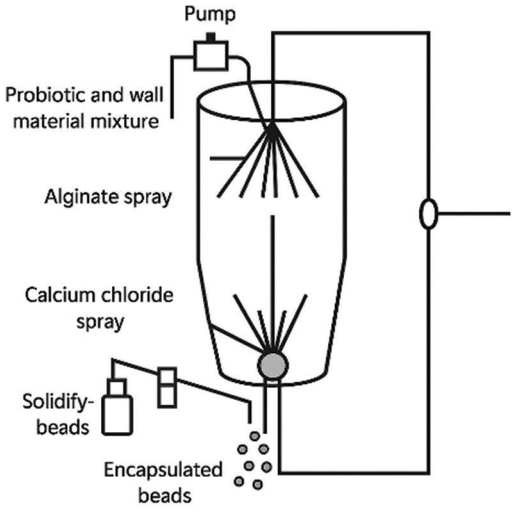


**Figure 11:** Encapsulation by the impinging aerosol technique

uniformly.[119] Compared to alternative drying methods, this technology delivers superior product structure and great persistence of volatile and bioactive chemicals.[120] Because of their very porous surfaces, spray-freeze-dried probiotic powders are useful for making matrix-type micro-capsules

that can be utilized with other industrial drying techniques to improve probiotic protection while being stored.[121] However, compared to spray drying, this technology is more expensive.

### Impinging Aerosol Technique

The process of impinging aerosol is straightforward, continuous, and scalable. It was created to generate tiny beads that are below 50 µm. It makes use of two distinct aerosols: one containing calcium chloride and the other containing a microbial suspension in alginate solution. The aerosols, calcium chloride, and alginate solution are injected via the top and bottom of the cylinder, respectively. This method is appropriate for encapsulating heat-sensitive and solvent-sensitive materials and was developed to lower sensory detection limits. The impact of impinging aerosol technologies on probiotics has been examined by several writers. Comparing the impinging aerosol technique to extrusion technology, the encapsulated *L. rhamnosus* GG demonstrated comparable survival rates and superior cell protection.[122] Emulsification, freeze drying, spray drying, and extrusion are some of the methods listed above that are frequently used to encapsulate probiotics. Spray drying, on the other hand, is anticipated to be a successful and alternative.

technique for producing probiotic powders on an industrial scale due to its low end product moisture content, low specific energy consumption (10 times lower than freeze drying), high reproducibility, short drying time, ready scalability, high process yield, suitability for thermolabile materials, and ability to protect probiotic strains from harsh environmental conditions effectively.[123]

### Fluid Bed Coating Technique

The fluidizing air stream used in fluid bed coating creates a consistent flow of feed particles within the processing chamber. It is among the most widely utilized particle coating processes. Atomising feed liquid to create a thin spray into a bed of fluidized particles is the fundamental idea behind fluid bed coating [Figure 12].[124] A chosen coating material is atomized using a nozzle and then allowed to harden at a low temperature or through solvent evaporation. To get a uniform coating, the particles inside the chamber must circulate properly. The probiotics are encapsulated using a variety of coating materials; however, fluid bed coating is more frequently done with lipid-based coating materials.[125] This process's requirement for huge amounts of powder to charge the plant is one of its drawbacks; therefore, the components should have simple shapes to prevent powder entrapment.

### Spray Cooling Technique

Like spray drying, spray chilling is a continuous, low-cost method that can be used for manufacturing on an industrial

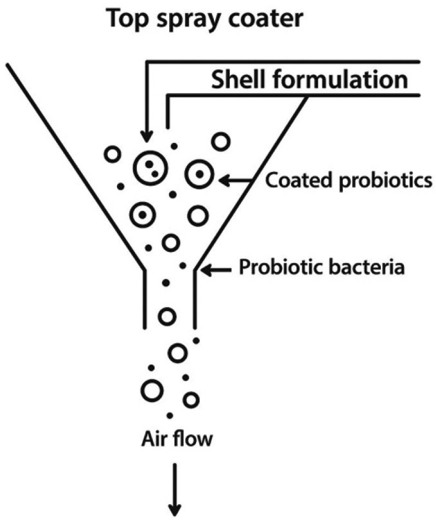


**Figure 12:** Encapsulation by fluid bed coating

scale.[126] The pharmaceutical industry uses it extensively to preserve enzymes, proteins, minerals, and flavours. It is also known as spray congealing.[127] This method primarily involves atomising bioactive substances that have previously been introduced to the carrier materials, typically lipophilic compounds, into tiny droplets. Injecting cold air solidifies these droplets.[128] The primary benefit of spray freezing is that it eliminates the need for high processing temperatures and organic solvents. High melting point carriers (45 and 122°C) are always used in the spray cooling process to produce spray particles, whereas low melting point carriers (32 and 42°C) are used in the spray chilling process. The energy flow direction is the primary distinction between spray cooling and spray drying [Figure 10]. Minimal encapsulation efficiency, release of active ingredients during storage, and limited applicability due to the hydrophobic nature of the particles are some drawbacks of this approach.

### Electro Spraying Technique

Electrospinning (electron + spinning) is a very flexible approach that combines the use of two methods, namely electrospray and spinning. This method involves applying a strong electric field to a fluid, which could be a melt or solution, that emerges from the tip of a die, which serves as one of the electrodes. As a result, the droplets distort, and eventually a charged jet is ejected from the tip in the direction of the counter electrode, creating continuous fibres.[129] The generation of extremely thin fibres or capsules with huge surface areas, a few nanometers, is one of the benefits of the electrospinning technology. Furthermore, this approach is highly appealing for a wide range of applications due to its simplicity and potential for large-scale outputs.[130] In addition, this process has certain drawbacks, such as restricted use, regulatory concerns, and the need for a high-voltage power source.

### Freeze Drying/Lyophilization

Probiotic powders have been made using freeze drying for many years; however, the idea of combining freeze drying and encapsulation is relatively recent. The three stages of the process—freezing, primary, and secondary drying—are based on sublimation. Cells are usually sublimed under a high vacuum after being frozen.[131] Higher probiotic survival rates are usually attained because freeze drying involves softer processing conditions than spray drying.[132] This method involves freezing the solvent and using sublimation to remove it.[74] When a molecule accumulates sufficient energy to separate from the molecules surrounding it, sublimation takes place. Sublimation (vacuum sublimation) occurs at temperatures ranging from -50 to -30 degrees Celsius and pressures between 0.05 and 0.1 millibars. Probiotic cell membrane damage results from ice crystal formation and stress conditions caused by osmolarity during freezing. Probiotic microorganisms are thereby stabilized and preserved using a variety of cryoprotectants, including lactose, sorbitol, sucrose, trehalose, skim milk, and milk protein.[133]

### Vacuum Drying

A third of the price of a freeze dryer, Hoover drying is an inexpensive method that works effectively for food items susceptible to heat. It operates by applying a vacuum during the drying process, which causes the food material's moisture to evaporate at low temperatures. Because there is less pressure in the chamber for drying, the water in the food evaporates more quickly than it would during regular drying.[134] To boost the survival rate of vacuum-dried Lactobacillus para-casei and Lactobacillus helvaticus, a variety of protectants, including sorbitol and trehalose, were employed. Lactobacillus paracasei vacuum-dried cells treated with trehalose exhibited a higher survival rate when held at 4°C; however, a lower stability was noted when stored at room temperature. Cell viability was not significantly reduced when sorbitol was applied to vacuum-dried cells and kept at 20°C. However, there are several drawbacks to vacuum drying, such as a lengthy processing time, batch processing, low efficiency, substantial running costs, etc.

## CONCLUSION

The intricate relationship between the human microbiome and overall health underscores the significance of maintaining a balanced microbiota. Research indicates that probiotics and prebiotics can provide substantial health benefits, particularly when combined effectively. The development of microencapsulation techniques represents a promising avenue for enhancing the efficacy of probiotics by ensuring their survival through the GIT and promoting targeted delivery to the colon. As our understanding of colon physiology and microbiota interactions continues to grow, innovative strategies for probiotic application can be expected to significantly improve health outcomes. Future research should integrate pharmaceutics, material sciences, biotechnology, and nanotechnology, focusing on refining these techniques and exploring their therapeutic potential in a broader range of diseases associated with microbiota imbalance.

## AUTHORS' CONTRIBUTIONS

FN conceptualized the review, researched the literature, and drafted the initial manuscript. MH provided technical support, especially in outlining the design, and assisted in formatting and finalising figures and tables. RS contributed to data collection and organization of encapsulation strategies. AP reviewed the microencapsulation techniques and contributed to writing and editing that section. All authors read and approved the final manuscript.

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