Introduction

The successful oral delivery of live probiotic bacteria to the gastrointestinal (GI) tract must adhere to a set of parameters similar to those faced by orally administered pharmaceuticals. The stability of a drug compound within a dosage form and the drug's oral bioavailability are critical parameters to consider when applying a delivery system to any pharmaceutical product. An analogous set of parameters – stability within a dosage form and viability after release – also applies to the administration of probiotic bacteria.

These parameters are dependent on both the design of the dosage form itself and the *in vivo* environment of the GI tract. The stability of a solid oral dosage form must encompass its production, including raw materials and method of manufacture, and the *in vivo* stability of its delivery system. Passage through the GI tract will subject the dosage form to large fluctuations in pH and erosional forces that will not only impact the viability of the bacteria contained within the dosage form, but will also affect the release pattern of the bacteria into the GI tract.

The degree to which the dosage form is resistant or adaptable to these changes in physical and chemical forces is a reflection of the robustness and ruggedness of the delivery system. An efficacious system will allow for the protection of the bacterial contents from exposure to acidic gastric fluid and prevent unduly rapid deterioration of the dosage form. A robust and rugged system will buffer stresses encountered during the manufacturing process, yield reproducible performance of the release profile and the net number of viable bacteria contained within the dosage form.

Dosage Form Design

The design of a dosage form is critical to its functionality as a vehicle for the oral delivery of probiotic bacteria. The majority of probiotic bacteria introduced into solid oral dosage forms are produced as lyophilized (freeze-dried) powder, and the manufacturing process must consider the exceptional characteristics and behavior of these lyophilized products. Poor flow properties and extreme sensitivity to heat, moisture, and air are hallmarks of lyophilized powders and necessitate special considerations during manufacture. The reactivity of lyophilized powders with other excipients must also be evaluated to ensure that fillers, controlling excipients and coating compounds do not unduly affect the reconstitution of the lyophilized bacteria or their post-reconstitution viability.

One such area of reactivity is available water, the amount of moisture a material is capable of absorbing from the surrounding environment. The available water present in the carriers, controlling excipients, and flow agents that are combined with the lyophilized powder to manufacture the dosage form may be a source of moisture sufficient enough to result in the premature reconstitution of lyophilized bacteria or oxidative damage to the cell membrane that inhibits future reconstitution.

Premature reconstitution from a lyophilized state causes the organisms to begin metabolizing available sources of energy; the constituents of the delivery system provide very limited sources of energy and when these locally available sources of energy or food

are exhausted, the organisms expire. The metabolic byproducts of prematurely reanimated organisms may also have a negative impact on the viability of the remaining, non-reanimated organisms. Oxidative damage to the cell membrane occurring during lyophilization, dosage form manufacture or storage also prevents reconstitution of otherwise viable bacteria due to their inability to regulate water uptake during the reconstitution process.

Dosage Form Selection

The type of solid oral dosage form, capsule or tablet, employed to orally administer the bacteria establishes the stresses experienced by the bacteria both during the manufacturing process and once it resides within the final dosage form. Moisture, air, heat, and compression are four of the most significant stresses. While no manufacturing process is immune to losses in viability due to any one form of stress, encapsulation is primarily affected by moisture, whereas tableting is more affected by heat and compression. Traditionally, capsules have been the preferred dosage form due to the lower manufacturing loss, and the adaptability of capsules to enteric coating and other gastric bypass methods. However, it should be emphasized that some degree of viability loss is associated with all methods of manufacture relative to the viability of free flowing lyophilized powder reconstituted in a controlled (laboratory) environment.

Encapsulation and tableting each involve very different stresses inherent to their specific process of transforming the lyophilized powder into a dosage form. The gelatin from which two-piece hard shell capsules are formed contains 12-18% moisture; moisture content below this range results in a brittle capsule and moisture content above this range allows the capsule to be too pliable, both extremes of malleability are unsuitable for manufacture. The extremely hygroscopic nature of the lyophilized powder allows the moisture present in the capsule to be osmotically drawn from the shell into the powder, which may result in reconstitution of the lyophilized bacteria prior to dosing, lowering the *in vivo* viability of the dosage form.

The permeability of a gelatin capsule may also result in moisture being taken up from the environment and conducted through the capsule to the lyophilized powder within; the permeability of gelatin capsules may also allow air to be transmitted through the shell and react with the lyophilized contents. The presence of significant quantities of moisture has been somewhat addressed through the use of coating technologies to reduce the permeability of the capsule shell and selective packaging methods, such as blister packing or inclusion of desiccants. The moisture present within the gelatin capsule itself can never be completely removed without causing the dosage form to become excessive brittle.

In contrast to capsule moisture and permeability however, tableting technologies must address the range of forces experienced by a powder during tableting. All forms of simple tableting generate some degree of heat and compression stress in the transformation of a free flowing powder to a solid tablet, which may have an adverse effect on the viability of lyophilized bacteria. The degree of viability loss due to these stresses varies widely; the compression characteristics of a given powder and the tableting conditions of a specific tablet press determine the extent of stress experienced by the bacteria. One means of circumventing this decrease in viability during production has been the addition of carrier compounds to the lyophilized bacterial powder.

These carrier compounds may act as cushioning and flow agents, reducing the heat and force of compression experienced by the bacteria, allowing for increased viability.

A significant advantage of a tableted dosage form is its resistance to moisture permeability. The degree of resistance varies depending upon the tablet constituents and hardness, through tablets are generally less susceptible to moisture permeation than capsules due to the greater density of the tablet interior and the lower permeability of the outer layer of the tablet. Hygroscopic excipients or softer tablets may increase moisture conductivity, harder tablets or less-hygroscopic excipients may decrease moisture conductivity.

Gastric Bypass

The most severe gradient in pH fluctuation a dosage form will encounter occurs in the transition from the acidic environment of the stomach to the more neutral environment of the small intestine. The normal range of pH for gastric fluid is 1.0 - 1.5 in healthy individuals; this pH will vary slightly depending on fed- or fasted (full or empty) conditions. The normal range for pH in the lower GI is 4.5 to 7 for the small intestine and 6.5 to 8 for the large intestine. The duration for residence within the gastric environment ("gastric residence time") is also dependent on fed or fasted conditions, ranging from less than an hour in the fasted state to up to two hours in the fed state. Thereafter, a dosage form's transit time through the small and large intestines occurs over 12-24 hours.

Within the pharmaceutical industry, dissolution testing is widely used to determine the effects of fluctuations of pH and gastric motility the release rate of a compound from a dosage form. This testing may be adapted for probiotic dosage forms to determine the effects of pH fluctuation on the release rate and viability of bacteria contained within a dosage form. The conditions of the exam should represent the longest duration of exposure to gastric fluid as would exist within the fed state, so as to ensure the robustness of the delivery system and the functionality of the dosage form under physiologic conditions. Dissolution testing performed using acid exposure durations of less than two hours may not reflect the behavior of the dosage form within the body while in the fed state. Examination of dosage form release and viability in the fasted state is possible using a shorter duration of acid exposure, but should always be conducted in combination with fed state examinations for the most accurate description of *in vivo* behavior.

The United States Pharmacopoeia (USP) describes a method of examining the effectiveness of enteric coated dosage forms and their behavior using media representing gastric and intestinal fluids. Although not all probiotic dosage forms are enteric coated, the method serves as a sufficient test of the robustness of any probiotic dosage form exposed to gastric fluid. Using a Type II dissolution apparatus at 50rpm and 37°C, the dosage forms should be placed in 1000mL 0.1N hydrochloric acid (pH 1.2) for 2 hours, and subsequently transferred to a buffered medium (pH 6.8) for the remainder of the dissolution period, (USP 25,724; Method B). The buffer solution prescribed by the USP, 0.2M tribasic sodium phosphate, is specific to pharmaceutical dosage forms and is not appropriate for the measurement of viable bacteria released from the dosage form.

Probiotic dosage forms lacking delivery systems and those dosage forms designed to dissolve upon contact with gastric fluid display an "immediate release" profile. Immediate release dosage forms disintegrate rapidly, often releasing the entirety of their bacterial payload contents within the stomach. While the degree of acid resistance varies among bacterial strains, exposure to an acidic environment detrimentally impacts the viability of most bacteria.

"Gastric protection," a delayed release profile minimizing of the bacteria's exposure to acid, is therefore one of the most significant uses of a probiotic delivery system, physically or chemically isolating the bacteria from the acidic medium of the upper GI. Several traditional mechanisms have been employed to achieve gastric bypass: enteric coating of the dosage form, microencapsulation of the lyophilized bacterial powder, and acid-resistant carrier compounds blended with lyophilized powder. The efficiency and the intricacy of production of each mechanism of delivery vary; higher manufacturing costs being associated with increasing complexity of production.

Enteric coating of a dosage form typically entails a coating procedure after the dosage form has been produced in its most basic form as an uncoated capsule or tablet. The dosage form is spray-, or pan-coated with one or more layers of a material that will selectively degrade when exposed to a fluid with a higher pH than the gastric environment allows. The coating must function as an effective barrier to gastric fluid – if the coating resists dissolution but allows penetration of acidic media, bacterial viability within the dosage form is often dramatically reduced. In addition, if the enteric coating exhibits surface damage or uneven thickness, premature breeching of the capsule contents is likely to occur. Preventing such damage often necessitates use of specialized packaging methods such as blister packing.

The process of coating a dosage form often involves use of an aqueous or solvent-based coating compound, which may introduce an additional loss of viability due to trace amounts of moisture or solvent contacting the lyophilized bacteria and the heat involved during the application and drying process. Because enteric coating compounds are selected for their ability to dissolve in the presence of non-gastric pH, their release profiles often display rapid dissolution times and the explosive release of the bacterial payload soon after transiting to the more neutral pH region of the duodenum and small intestine. This rapid dissolution effectively mimics the performance of an immediate release delivery system once beyond the gastric environment, delivering the entirety of its contents to a single location and relying upon natural peristalsis (contractions of the GI muscles) and intestinal fluid dynamics to move the bacteria distally away from the point of release.

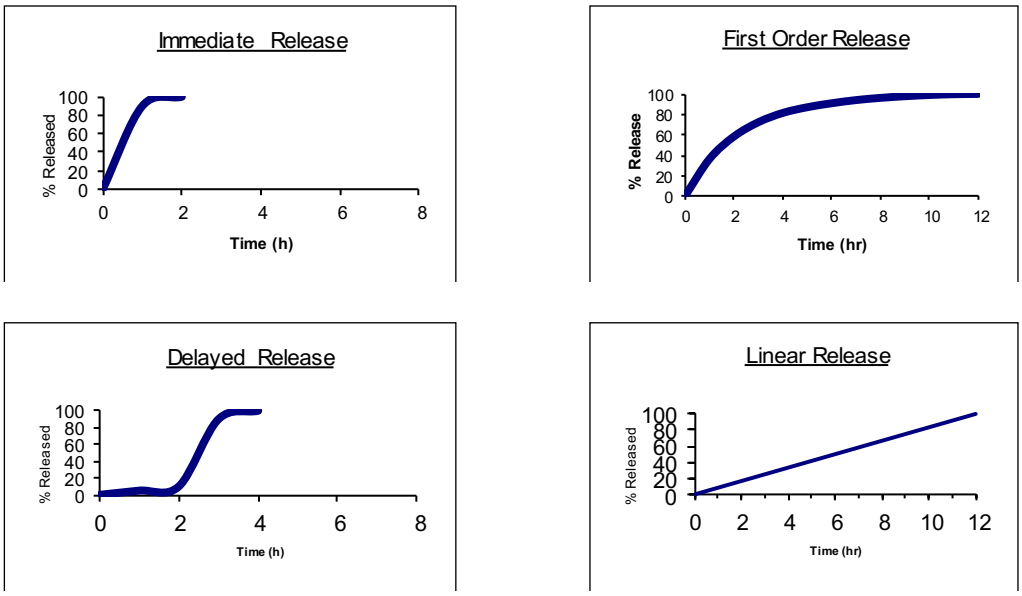
Micro-encapsulation of lyophilized bacteria and the use acid resistant carrier compounds have also emerged as functional alternatives to enteric coating. Micro-encapsulation is often achieved through phase-separation of carrier-lyophilized bacteria oil/water emulsions, and collection of the microencapsulated beads through a series of centrifugation, wash, and sizing procedures. The encapsulating compound may be a hydrophobic compound functioning to exclude water from the lyophilized interior of the particle, a probiotic compound to facilitate post-reconstitution viability, or may have a pH-sensitive or time-release coating to allow for gastric bypass delivery. The blending of acid resistant carrier compounds with lyophilized bacteria may be accomplished in a

variety of ways, via dry granulation or dry-blending prior to loading into a capsule or compression into a tablet. While effective, both technologies involve multiple manufacturing processes prior to final dosage form manufacture, significantly increasing production costs compared to simple encapsulation or tableting methods. Micro-encapsulated beads require uniform coating thickness and bead sizes to produce uniform behavior *in vivo*, and like enteric coatings, are subject to viability loses resulting from coating flaws and manufacturing stresses.

Controlled-Release Probiotic Delivery

An alternative to delivering probiotics using traditional immediate release and delayed release-gastric bypass systems is the use of a "controlled release" delivery system that provides not only gastric bypass, but continuous release of the bacterial payload over an extended duration. Controlled release ("controlled delivery") technologies have been widely used in the pharmaceutical field to achieve more therapeutically beneficial release profiles, reductions in side-effects and more convenient dosing regimens. The capability of sustaining the release of a drug from a single dosage form for 12-24 hours, allowing once-per-day or twice-per-day dosing, has substantially improved patient compliance in many drugs that previously required multiple doses throughout the day.

Applications of controlled release technologies may present similar advantages to the delivery of probiotic bacteria: extended release over several hours, improved therapeutic efficacy, and a more convenient dosing regimen for the consumer. The release profile of a controlled delivery system may be designed to release in a specific pattern. "Near-linear" or "zero-order kinetics" profiles may release bacteria from the dosage form at a near-constant rate. "First-order kinetics" profiles may initially release bacteria more quickly during the first few hours of dissolution, followed by a period of slower release. Such profiles allow for a continuous release of bacteria throughout the gastrointestinal tract; a minimal amount of release occurs in the upper GI for purposes of stimulating immune response, with the vast majority of viable release occurring beyond the gastric environment.

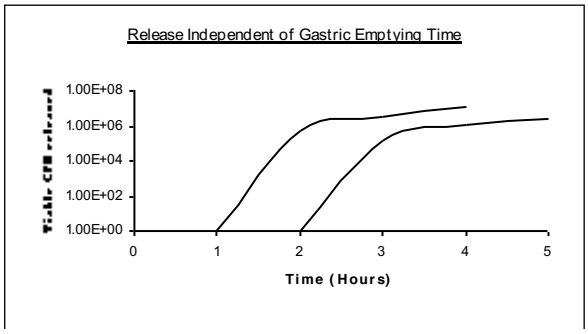


Standard platforms for pharmaceutical delivery are not well suited to the delivery of live organisms such as probiotic bacteria. Historically, the application of pharmaceutical controlled release technologies to probiotics has been limited by factors of cost and viability loss. Classic pharmaceutical delivery systems such as osmotic-pump and physical-geometric systems require complex manufacturing processes and have costs greater than those supportable by the present probiotic market. The incorporation of lyophilized bacteria into simple matrix and hydrogel-diffusion systems are hampered by the stresses imparted during manufacture and their inability to provide the capability for gastric bypass without the use of coating processes or chemical modifications potentially toxic to the bacterial dose.

The lack of controlled delivery technology applicable to the probiotic market prompted the development of a novel, cost-effective system capable of delivering lyophilized bacteria in a controlled-release manner. Nutraceutix's BIO-tract® delivery technology is the first system capable of delivering probiotic bacteria in a true controlled release manner. This controlled release delivery system allows for the continuous release throughout the dissolution period, while also achieving gastric bypass.

BIO-*tract*® provides a means of attaining controlled delivery of probiotic bacteria from a modified diffusion-erosion matrix tablet. The modified diffusion-erosion matrix is only activated *in situ*, produced as a monolithic tablet and existing as a stable, monolithic tablet prior to hydration. The tablet itself is formed from a simple two step-process of dry blend and direct compression. The viability lost due to stresses of tablet manufacture is minimized through the use of selected cushioning carriers and flow agents, and is less than or equal to losses experienced in conventional tableting procedures. The granulation, coating and other processing steps required for more complex pharmaceutical systems to achieve gastric bypass are unnecessary because the

Intra-dosage form pH is modulated through the use of internally buffering electrolytes. The ability to achieve gastric bypass without the use of enteric coating also allows for the technology to be relatively independent of food effects such as gastric emptying time.



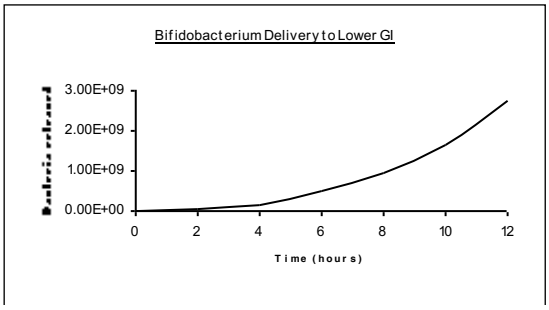
The release of bacteria is mediated by a combination of erosion of the tablet excipients and diffusion of the bacteria from the tablet matrix. The initial hydration of the tablet occurs upon contact with the fluid present in the stomach. As the surface of the tablet hydrates, the polymeric matrix swells and forms a barrier to further fluid permeation. As the swollen region of the tablet erodes over time, and fluid penetrates progressively toward the core of the tablet, the bacteria within the hydrated region of matrix are reconstituted and released.

The processes of hydration, swelling and erosion of the polymeric matrix and the reconstitution and release of the bacteria contained within the matrix occur simultaneously. The highly segregated regions of polymer hydration within the tablet provide distinct regions in which bacteria in differing states of reconstitution exist. The fully hydrated region near the surface of the tablet contains reconstituted bacteria. The semi-hydrated interior contains both reconstituted and dormant bacteria, the proportion of reconstituted bacteria being greater in the region in closest proximity to the areas of fluid penetration. The unhydrated core of the tablet contains wholly unreconstituted, lyophilized bacteria.

The distinct regions of tablet hydration also provide a gradient of osmotic stress experienced by the lyophilized bacteria during the process of reconstitution. This contrasts with the dramatic change in osmotic pressure experienced by bacteria explosively released from immediate and delayed release dosage forms. The physiological effects of such a sharp change in osmotic pressure may lower their viability during reconstitution. While this gradient is most pronounced in the semi-hydrated regions of the tablet, it also occurs in the interface between the semi-hydrated region and the unhydrated core, and the boundary region near the surface of the tablet. Lyophilized bacteria near the surface of the tablet are rapidly reconstituted during the initial hydration that occurs in the stomach. As the dosage form transits through the intestinal tract, the matrix progressively hydrates and the polymers slowly disentangle, allowing fluid to penetrate and reconstitute the lyophilized bacteria present. The rate of disentanglement and fluid penetration within the tablet is such that the osmotic pressure of the microenvironment surrounding the yet unreconstituted bacteria reduces the impact of osmotic stress on their viability.

Tablet hydration and polymer disentanglement occur continuously throughout the dissolution time period; bacteria within different regions of the dosage form will be exposed to the differing ranges of gastro-intestinal pH according to the location of the dosage form as it transits through the intestinal tract. The bacteria near the surface of the tablet being exposed to acidic fluid penetration and the bacteria within the interior of the tablet exposed to the more neutral pH of the intestines. The homogeneous distribution of electrolytes within the dosage form allows for a relatively constant pH to be maintained in the hydrated regions of the tablet, (the unhydrated regions of the tablet not being exposed to any fluid and therefore not subject to changes in gastrointestinal pH). Although the initially hydrated region near the surface of the tablet is exposed to gastric fluid of an acidic pH, the pH within that region will be mediated by the presence of the electrolyte. Within the semi-hydrated interior of the tablet, this mediating effect is more pronounced, with the hydrated region reflecting the pH of the hydrated electrolyte. This phenomenon allows for an electrolyte to be selected for an intra-dosage form pH specific to the optimal reconstitution pH for a given bacterial strain being delivered.

The rate of tablet hydration and bacterial release is programmable through altering the ratios of individual excipients within the dosage formulation. Polymers of greater viscosity may extend the duration of release, while the inclusion of controlling excipients whose dissolution characteristics are dependent on pH occurring within a fixed region of the GI tract allow for the rate of release to be altered accordingly. This flexibility in release rate allows for the capacity to design release profiles specific for the attachment location of a given bacterial strain. Such strain-specific release profiles might include the delivery of *Lactobacillus Acidophilus* to the small intestine, or the delivery of *Bifidobacterium Bifidum* to the lower GI.



The first embodiment of the BIO-*tract*® technology is Nutraceutix's BIO-*tract*®, a controlled release, multiple-strain, gastric-protection delivery system. BIO-*tract*® employs conventional excipients and a simple dry-blend, direct-compression manufacturing process to create a cost-efficient monolithic tablet capable of delivering bacteria throughout the length of the gastrointestinal tract. The polymeric formulation ensures a consistent rate of matrix erosion and excellent gastric-bypass performance under fed or fasted conditions. Relative independence from food effects such as gastric retention time and an extended release profile provides the consumer with the convenience of dosing without regard to meals. It should be noted that while BIO-*tract* is ideally suited for the delivery of probiotic organisms due to their susceptibility of damage from exposure to stomach acids, other active ingredients that have the same characteristics may also find BIO-*tract* to be highly applicable.

The excipients contained in the formulation as recognized as being G.R.A.S. (Generally Regarded As Safe), and are wide available within the industry. Hydroxypropyl methylcellulose, a swellable polymer employed widely throughout the pharmaceutical industry, and apple pectin, a polysaccharide whose dissolution characteristics are dependent on the pH and enzymatic conditions present in the lower GI tract, are employed as the chief controlling excipients. $NaHCO_3$ is a salt that functions as an electrolyte, providing a neutral intra-dosage form pH. Microcrystalline cellulose, stearic acid, and silica dioxide perform as carriers, cushioning agents, flow agents and desiccants.

The delivery system demonstrates excellent robustness and ruggedness, achieving consistent levels of gastric bypass and controlled release while displaying good manufacturing characteristics such as powder flow and tablet throughput. The dry-blend, direct-compression manufacturing process is identical to that of an uncoated, immediate release dosage form. The manufacturing loss in bacterial viability resulting from heat and compression forces to create the tablet is less than or equal to conventional tableting processes, such as employed to create Nutraceutix's LiveBac® technology. Because the dosage form is a monolithic tablet, BIO-*tract*® is amenable to a variety of packaging options, including single-dose foil sachets and HDPE (high-density polyethylene) bottles with desiccants.

United States Patent # 8,007,777
Australia # 2002330187
Canada # 2,461,708
Singapore # 103611
Japan # 5,041,651
Republic of Korea # 10-0979877
China (People's Republic) ZL 02823678.5
Russian Federation #2,313,355
European Patent Convention 1429802
(*Austria, Belgium, Bulgaria, Switzerland, Cyprus, Czech Republic, Germany, Denmark, Estonia, Spain, Finland, France, United Kingdom, Greece, Ireland, Italy, Lithuania, Luxembourg, Monaco, The Netherlands, Portugal, Sweden, Slovakia, Turkey*)