

W4200 Novel Design of a Monolithic Oral Controlled-Release Delivery Formulation for Probiotic Organisms

S. J. Turner¹, M. Hite², C. Federici² ¹Product Development, ²Research & Development, SCOLR, Inc., Redmond, WA 98052

Purpose. To design a novel monolithic, solid oral dosage form capable of protecting tableted live organisms through the stomach and delivering these organisms in a sustained fashion in the lower gastrointestinal tract. **Methods.** Several formulations were designed using common pharmaceutical excipients and active lyophilized bacteria. The formulations were designed to be manufacturable as directly compressible dry blends, avoiding additional costly processing steps. Tablet hardness and friability and blend powder characteristics were examined to ensure ease of large-scale manufacture. Surrogate *in vitro* dissolution tests were developed to more closely approximate the environmental changes the tablet would experience during transit *in vivo*. Live CFUs from samples taken at relevant timepoints were examined to establish gastric survival rates. Intragastric pH was monitored as hydration occurred to ensure a neutral environment surrounding the organisms before release from the dosage form. **Results.** The formulation components and excipients chosen effectively control the rate of hydration at lower pH, maintaining a neutral microenvironment within the tablet core and minimizing organism die-off. The formula may be altered to exhibit the desired release profile as well as ensure a relevant amount of gastric survivability. **Conclusions.** Results show that readily manufacturable formulations of living organisms ensuring significant gastric survivability and delivery to the lower gastrointestinal tract are possible using this novel monolithic delivery system.

Introduction:

The objective of this project was to create a novel monolithic, solid oral dosage form capable of delivering lyophilized bacteria in a sustained fashion throughout the gastrointestinal tract. This dosage form should prevent excessive losses in bacterial viability during gastric residence and demonstrate a reproducible controlled release profile. The dosage form should be manufacturable from a directly compressible, dry-blend, resulting in an uncoated monolithic tablet whose process of manufacture does not result in losses of bacterial viability greater than those experienced in the production of immediate-release dosage forms.

The formulations used to create the dosage forms were adapted from a polymeric matrix system developed to deliver highly soluble pharmaceuticals independent of the pH of the *in vivo* environment. The inclusion of soluble electrolytes within a simple hydrophilic polymer matrix allows for the maintenance of a relatively constant pH within the dosage form, while also providing a mechanism for the preservation of dissolution channels formed as the polymeric matrix swells due to the infiltration of water present in gastric and intestinal fluid. The fluid hydrating the polymeric matrix also serves to reconstitute the lyophilized bacteria present. As the matrix hydrates, an *in situ* reaction between the polymer and electrolyte allows for the formation of distinct regions of polymer hydration and bacterial reconstitution. The surface region contains fully hydrated polymers and wholly reconstituted bacteria, limited by a hardened boundary region that is resistant to both erosion and further fluid penetration. Progressive hydration toward the center of the dosage form results in a gel region of partially hydrated polymers and partially reconstituted bacterial populations, and a dry core of unhydrated matrix and unreconstituted bacteria at the dosage form's center. The gradual erosion and polymer disentanglement of the matrix results in the controlled release of the bacteria.

Materials & Methods:

Hydroxypropyl methylcellulose (HPMC) was purchased from Dow Chemical (Midland, MI). Pectin 150 Slow-Set was purchased from Pacific Pectin, Inc. (Oakhurst, CA). NaHCO₃ was purchased from Spectrum Chemical (New Brunswick, NJ). Na₂CO₃ was purchased from JT Baker (Phillipsburg, NJ). Microcrystalline cellulose 102 was purchased from Stauber Chemical (Fullerton, CA). Silica Dioxide was purchased from Degussa Ltd. (Macclesfield, Cheshire UK). Stearic Acid was purchased from Ashland Chemical, (Santa Ana, CA).

Preparation of tablets:

All constituents were dry blended with a minimum amount of flow agents and directly compressed using either a hydraulic single station press (Carver, Inc.

Wabash, IN) at 2 tons, or a 16-station rotary press (Stokes BB-2, DT Industries Hyannis, MA) at 1.5-2.5 tons.

In vitro release study:

Release studies were conducted using a USP 25 Type II dissolution apparatus (Erweka DT-70, Erweka Corp. Milford, CT) with a paddle speed of 50rpm and bath temperature of 37.0±/-1.0 C. All vessels and paddles were autoclaved prior to use; dissolution assembly was disinfected with 70% Isopropyl alcohol (VWR, Westchester, PA) prior to use and between media transfers.

For the determination of gastric survivability (gastric bypass efficacy), dissolution of the dosage forms was conducted in gastric media, and subsequently transferred to peptone media buffered at pH 6.8, stomached, and dissolved for approximately 1 hour at 220rpm to yield maximum dispersion for uniform sampling. Gastric media was 0.1N HCl or USP simulated gastric fluid (SGF) at pH 1.2 for 30min or 2 hours. Samples were removed via sterile pipette, diluted to appropriate volumes for enumeration and plated on DeMan Rogosa Sharpe Medium(MRS) or Reinforced Clostridial Agar/Medium (RCM) via standard microbiologic technique. The plates were removed and the enumeration of Colony Forming Units (CFU) was conducted after 48-72 hours incubation.

For the determination of release rate, dissolution of the dosage forms was conducted in gastric media as described above and subsequently transferred to peptone media buffered at pH 6.8 to continue dissolution, sampled hourly. At the endpoint of dissolution, dosage forms were stomached, and dissolved for approximately 1 hour at 220rpm to yield maximum dispersion for uniform sampling. Live CFU were enumerated as above; total bacteria released was determined using a fluorochrome stain, 4',6-diamidino-2-phenylindole (DAPI), and a direct count method adapted from Kepner and Pratt. An Olympus Automatic Photomicrographic System (PM-10ADS, Olympus America Inc, Melville, NY), Fujicolor ISO/ASA 200 speed film, (Fuji Photo Film, Inc, Greenwood, South Carolina) at Film Speed Setting 800, Reciprocity 4, and Exposure Adjustments 2.5-4.0 was used to photograph slides prepared from filtered samples.

For the determination of matrix erosion, dissolution of the dosage forms was conducted as described above. At the endpoint of dissolution, dosage forms were removed to a drying oven for overnight desiccation and subsequent weighing.

0.1N HCl gastric media was prepared from 8N HCl (Spectrum Chemical, New Brunswick, NJ) and dH₂O. USP Simulated Gastric Fluid (SGF) was prepared from 8N HCl, pepsin (Spectrum Chemical, New Brunswick, NJ) and Sodium Chloride (Spectrum Chemical, New Brunswick, NJ).

Dissolution in fed state conditions was conducted by coating the dosage form in USP mineral oil, (McKassen Corp, San Francisco, CA), prior to introduction into gastric media.

Results and discussion:

Figure 1 shows viable CFU remaining upon entry into the intestinal tract (post-gastric viability) for a variety of gastric media and gastric residence times. To examine the effects of differing durations of gastric residence, both fed and fasted conditions were investigated for a GRT of 120 minutes, while only fasted conditions were investigated for a GRT of 30 minutes. A gastric residence time of 30 minutes was used to simulate the quickest transit time through the gastric environment as might exist in the fasted state. A gastric residence time of 120 minutes was employed to simulate the longest duration of dosage form exposure to gastric conditions as might exist in the fed state or as might occur if the dosage form floats in the stomach. Viability was measured in both 0.1N HCl and USP SGF to ensure the presence of enzymes did not adversely affect the dosage form viability. Viabilities of a dosage form unexposed to acid, (Initial (t₀)), and a compressed dosage form of lyophilized powder, (control), were also evaluated.

As expected, GRT has a significant effect on the viability of bacteria within the dosage form, with longer durations of exposure to acidic gastric fluid resulting in lowered viabilities. GRT of 30 min showed only slight reductions in viability compared to the unexposed dosage form; dosage forms in 0.1N HCl and SGF media delivered 1.1E9 and 1.2E9, respectively, compared to 1.3E9 CFU present in the unexposed dosage form. The difference in the amount of viable bacteria delivered to the intestinal tract after GRT of 30 min and GRT of 120 min (fed conditions) was generally less than 0.5 logs in SGF and 0.65 logs in 0.1N HCl. The salt-polymer interaction retarding gastric fluid penetration and the presence of electrolytes to mediate intra-dosage form pH are thought to be the primary mechanisms that allow for this relatively small variation. Fed conditions resulted in higher viabilities in both 0.1N HCl and SGF, likely through hampering the permeation of gastric fluid through the tablet shell. In general, SGF consistently demonstrated a less-adverse effect upon bacterial viability than 0.1N HCl.

Figure 1. Viable CFU remaining upon entry into the intestinal tract (post-gastric viability).

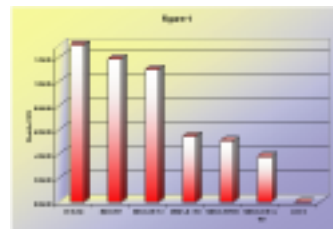
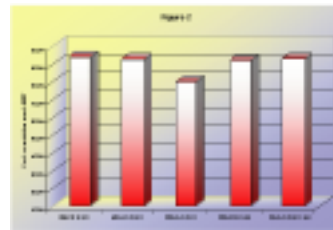


Figure 2 shows the weight remaining of the dosage form prior to entry into the gastrointestinal tract (erosion during gastric residence) for a variety of media and gastric residence times. The erosion of the matrix was measured to determine the consistency of the erosion process and the significance of acid penetration in the loss of bacterial viability during gastric residence prior to the erosion of the outer layer of the tablet.

Figure 2. Weight remaining of the dosage form prior to entry into the gastrointestinal tract (erosion during gastric residence).

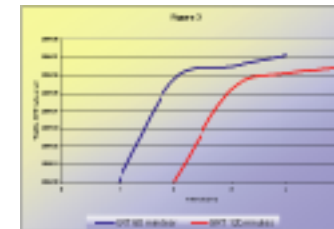


The amount of erosion experienced appears to occur most during the initial hydration phase, as shown by the loss of 6-7% of the dosage form weight during the first 30 minutes. The rate of erosion slows dramatically during the next 30 minutes, likely due to the formation of a hardened boundary region by the salt-polymer interaction. The second hour of GRT demonstrates the disentanglement and erosion of the boundary region, culminating in the loss of an additional 10% of the matrix. The lesser degree of erosion seen in fed conditions is likely due to the hampered penetration of fluid into the tablet, thus slowing the rates of hydration or disentanglement of the polymers within the hardened boundary region. Through a comparison with the data shown in **Figure 1**, the amount of erosion during GRT is shown to be less than the decrease in viability during GRT. This is presumably due to acid penetration during gastric residence occurring concomitantly with erosion; the dosage form was removed for desiccation prior to complete polymer disentanglement and erosion within the hydrated region.

Figure 3 shows the release pattern of viable CFU given different gastric residence times. The formulation employed was designed to deliver bacteria into the small intestine region. As described above, GRT affects both the viability of bacteria within the dosage form and the erosion of the dosage form itself. The effect of GRT on the release profile during the three hours following exit from the gastric environment was investigated using two residence times of 60 and 120 minutes

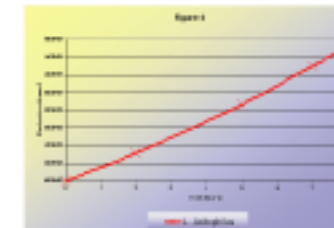
in 0.1N HCl. The pattern of bacterial release was similar for both 60 and 120min GRT, with 5-6% of the total CFU delivered in the first hour after introduction into non-gastric media. The effect of a longer period of hydration while in gastric residence is more apparent in the second hour, with 60min GRT creating a less hydrated dosage form releasing roughly 23% of the total CFU delivered while the more hydrated dosage form exposed to 120min of gastric media releasing 46% of the total delivered CFU. This variation in release due to differing degrees of matrix hydration was expected due to the longer duration of swelling and erosion within an acidic environment. Increasing the volume or viscosity of the polymeric matrix, or increasing the volume or solubility of the salt may aid in reducing the variability of bacterial release patterns with differing gastric residence times.

Figure 3 shows the release pattern of viable CFU given different gastric residence times.



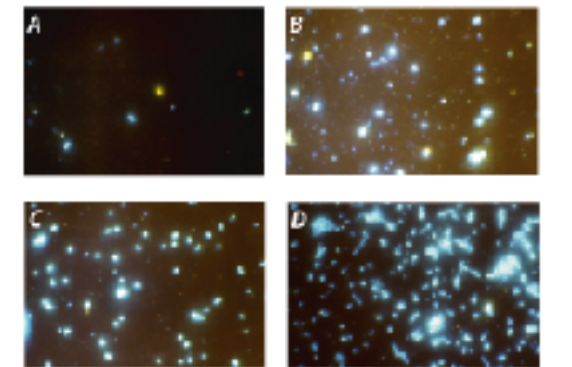
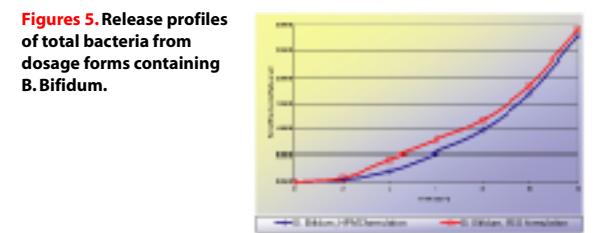
Figures 4 and 5 show release profiles of total bacteria from dosage forms containing *L. Acidophilus* and *B. Bifidum* formulations, respectively. Both the release profile and the intra-dosage form pH were selected to be strain-specific, delivering the majority of the bacteria within the region of attachment and providing a microenvironment conducive to the reconstitution of the lyophilized bacteria. *L. Acidophilus* preferentially attaches to regions of the upper GI tract and small intestine with their more aerobic, lower pH environments. *B. Bifidum* preferentially attaches to regions of the lower GI tract, in more anaerobic, higher pH environments. **Figures 6a-d** show a series of DAPI fluorochrome microphotographs used to directly enumerate samples comprising the release profile shown in **Figure 5**. **Figures 6a-c** correspond to samples removed at 2, 6, and 10 hours, respectively; **Figure 6d** shows a sample removed after allowing for maximum dispersion.

Figures 4. Release profile of total bacteria from dosage forms containing L. Acidophilus.



Figures 5. Release profiles of total bacteria from dosage forms containing B. Bifidum.

Figures 6a-d. Series of DAPI fluorochrome microphotographs. Used to directly enumerate samples comprising the release profile shown in Figure 5.



Conclusions:

The dosage forms created using this novel monolithic delivery system successfully delivered live bacteria to the intestinal tract without experiencing excessive loss of viability during the period of gastric residence. The dry-blend and direct-compression process of manufacture displayed satisfactory ruggedness and resulted in tablets of adequate hardness and friability. The finished dosage forms displayed reproducible patterns of erosion and profiles of release over extended durations.

The mechanics of the delivery system allow for development of strain-specific formulations capable of modulating intra-tablet pH specific to a given organism's preferred reconstitution environment and release profile.

References:

Fasshi and Pillay. A Novel Approach for the Constant Rate Delivery of Highly Soluble Bioactives from a Simple Monolithic System. *J. Contr. Rel.* 67 (2000):67-78
 Kepner and Pratt. Use of Fluorochromes for Direct Enumeration of Total Bacteria in Environmental Samples: Past and Present. *Microbiol. Rev.* Dec 1994. 58(4):603-615.

Citation:

Novel Design of a Monolithic Oral Controlled-Release Delivery Formulation for

Process	Actual Value	Target Value
A	1.1E9	1.3E9
B	1.1E9	1.3E9
C	1.1E9	1.3E9
D	1.1E9	1.3E9
E	1.1E9	1.3E9
F	1.1E9	1.3E9
G	1.1E9	1.3E9
H	1.1E9	1.3E9