Formulation Strategies for Solid Oral Drug Products to Prevent or Treat Diseases of the Gut Microbiome

Andrew Bristol, PhD

Vice President, Development

May 22, 2018

Forward-Looking Statements

This presentation includes forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, as amended, on Synthetic Biologics' current expectations and projections about future events. In some cases forward-looking statements can be identified by terminology such as "may," "should," "potential," "continue," "expects," "anticipates," "intends," "plans," "believes," "estimates," "indicates," and similar expressions. These statements are based upon management's current beliefs, expectations and assumptions and are subject to a number of risks and uncertainties, many of which are difficult to predict and include statements regarding our timeline for our SYN-004 (ribaxamase) and SYN-010 clinical trials and reporting of data, the size of the market, benefits to be derived from use of SYN-004 (ribaxamase) and SYN-010, our anticipated patent portfolio, and our execution of our growth strategy. The forward-looking statements are subject to risks and uncertainties that could cause actual results to differ materially from those set forth or implied by any forward-looking statements. Important factors that could cause actual results to differ materially from those reflected in Synthetic Biologics' forward-looking statements include, among others, our product candidates demonstrating safety and effectiveness, as well as results that are consistent with prior results, our ability to initiate clinical trials and if initiated, our ability to complete them on time and achieve the desired results and benefits, our clinical trials continuing enrollment as expected, our ability to obtain regulatory approval for our commercialization of product candidates or to comply with ongoing regulatory requirements, regulatory limitations relating to our ability to promote or commercialize our product candidates for the specific indications, acceptance of our product candidates in the marketplace and the successful development, marketing or sale of our products, developments by competitors that render our products obsolete or non-competitive, our ability to maintain our license agreements, the continued maintenance and growth of our patent estate, our ability to become or remain profitable, our ability to establish and maintain collaborations, our ability to obtain or maintain the capital or grants necessary to fund our research and development activities, a loss of any of our key scientists or management personnel, and other factors described in Synthetic Biologics' annual report on Form 10-K for the year ended December 31, 2016, subsequent quarterly reports on Form 10-Qs and any other filings we make with the SEC. The information in this presentation is provided only as of the date presented, and Synthetic Biologics undertakes no obligation to update any forward-looking statements contained in this presentation on account of new information, future events, or otherwise, except as required by law.

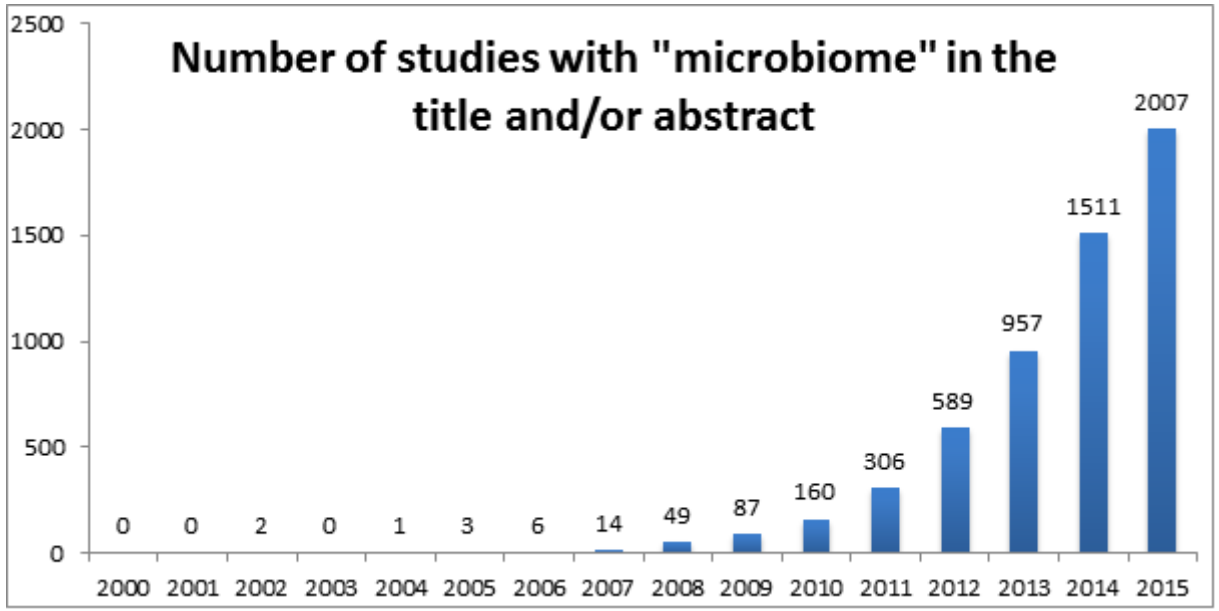
Our Mission

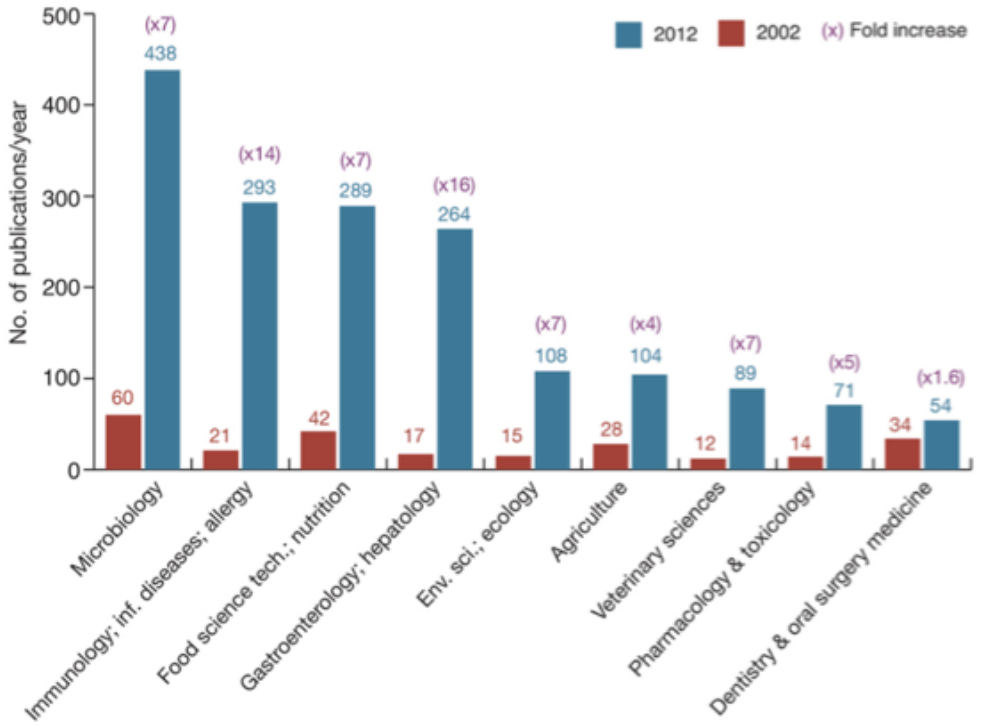
"Pioneering elegant solutions that leverage the microbiome to improve global health"

Presentation Outline

- Targeted, oral delivery of medicines to the gut microbiome
- Excipient and enteric protection selection
- Example 1: SYN-004 (ribaxamase), biological β-lactamase, for prevention of *C.difficile* infection (CDI)
- Example 2: SYN-010, small molecule lovastatin lactone, targeting *M.smithii*, for Irritable Bowel Syndrome with Constipation (IBS-c)

Microbiome Research & Development





Clinicaltrials.gov search term "gut microbiome":
349 clinical trials in US
1,317 clinical trials Worldwide

Oral Delivery of Biological Products to be Absorbed

Holy Grail: to deliver biological medicines systemically via oral route: No needles, no inhalers, etc.

Classic example: Insulin
First tried in humans in 1922, with poor results
Drug developers still trying, making limited progress

Barriers to absorption:
Physical:
- mucous layer
- intestinal epithelium
- tight junctions

Biochemical:
- low pH
- digestive enzymes (proteases)

Formulation:
- chemical modification (eg. PEG)
- co-administration with protease inhibitors, permeation enhancers
- microspheres, liposomes, nanoparticles

Oral Delivery of Biological Products to the Gut

Marketed oral enzymes and their excipients:

Beano® (α-galactosidase): mannitol, povidone, corn starch, water, sodium stearyl fumarate, polyvinyl acetate, less than 1% of: maltodextrin, silicon dioxide, calcium gluconate, triacetin, natural&artificial flavor, red no. 40 lake, acetic acid, sodium lauryl sulfate.

Lactaid® (lactase): Microcrystalline Cellulose, Croscarmellose Sodium, Crospovidone, Magnesium Stearate, Colloidal Silicon Dioxide.

Creon® (pancreatic replacement enzymes: lipase, protease, amylase): cetyl alcohol, dimethicone, hypromellose phthalate, polyethylene glycol, triethyl citrate.

Each of these are aids to digestion, but do not target the gut microbiome

Considerations for Targeted Delivery of Biological Drugs

Drug Product Release:

- pH at target region
- Time (GI transit)
- Osmotic pressure
- Enzymatic (chitinase)
- Combination of these

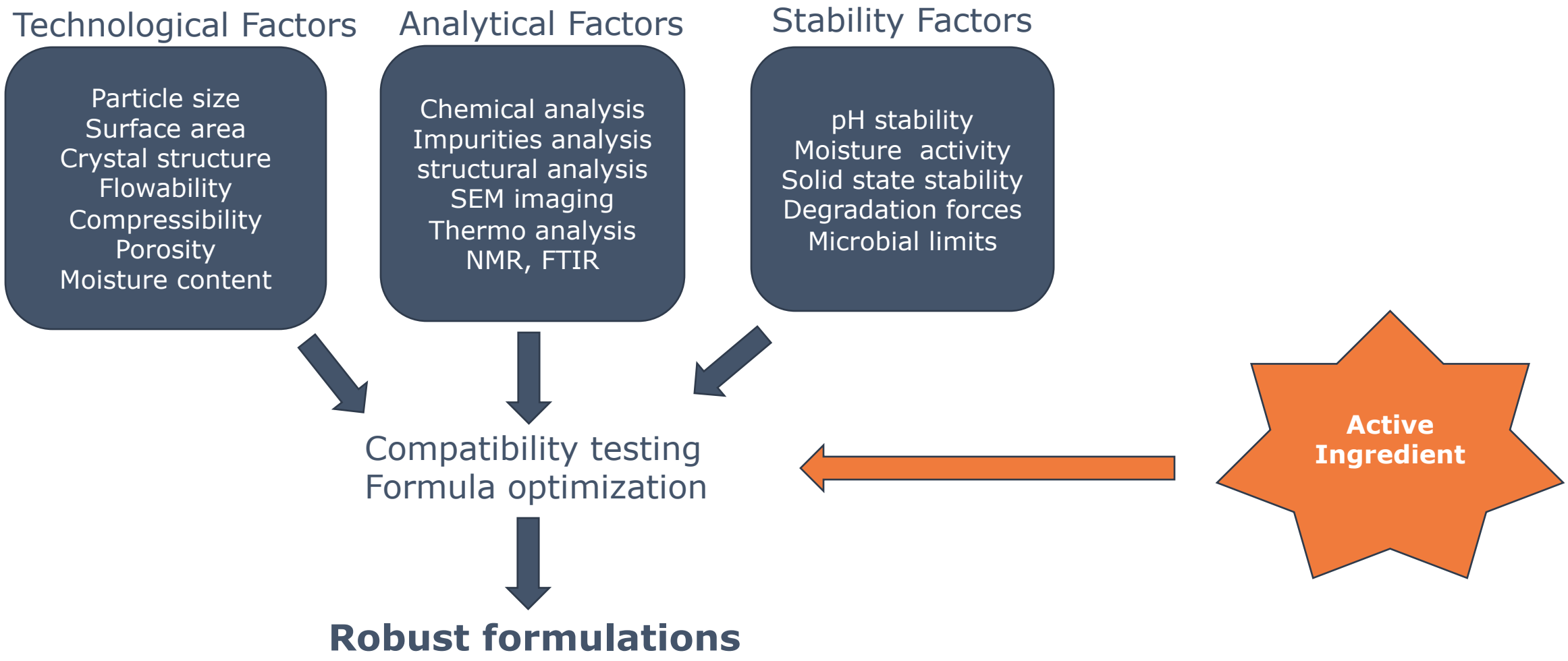
- Excipients can deliver via these mechanisms
- Compatibility with active ingredient

- Coated capsules, coated tablets, coated spheres, coated microspheres
  - Depends on indication, patient population, mfg. process

Drug Substance:

- Stability of chemical or biological: engineered for stability
- Solubility in intestinal environment
- Activity in intestinal environment: pH ~4.8 to 8.2
- Variability of target molecule/organism presence

Pharmaceutical Excipients: Properties and Testing



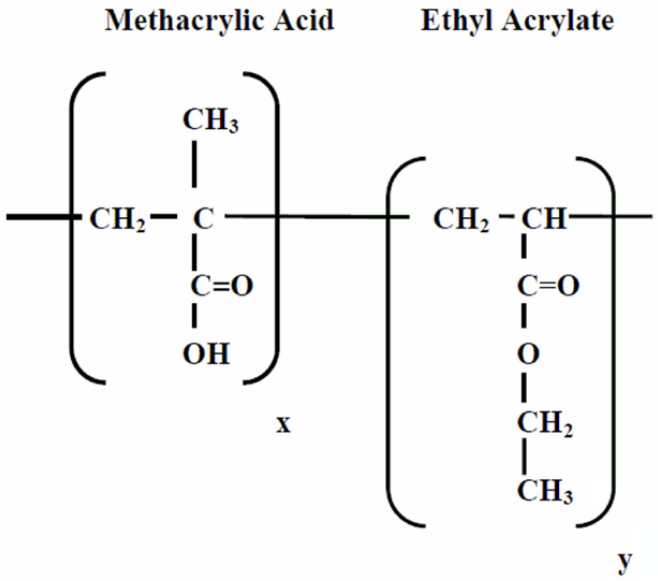
Enteric Coating Polymers to Target Desired Release Profile

Enteric protection:

Eudragit, based on methacrylic acid polymers

- Stomach
- Duodenum
- Jejunum
- Ileum, colon

HPMC phthalate
Ethylcellulose
Polyvinyl acetate phthalate



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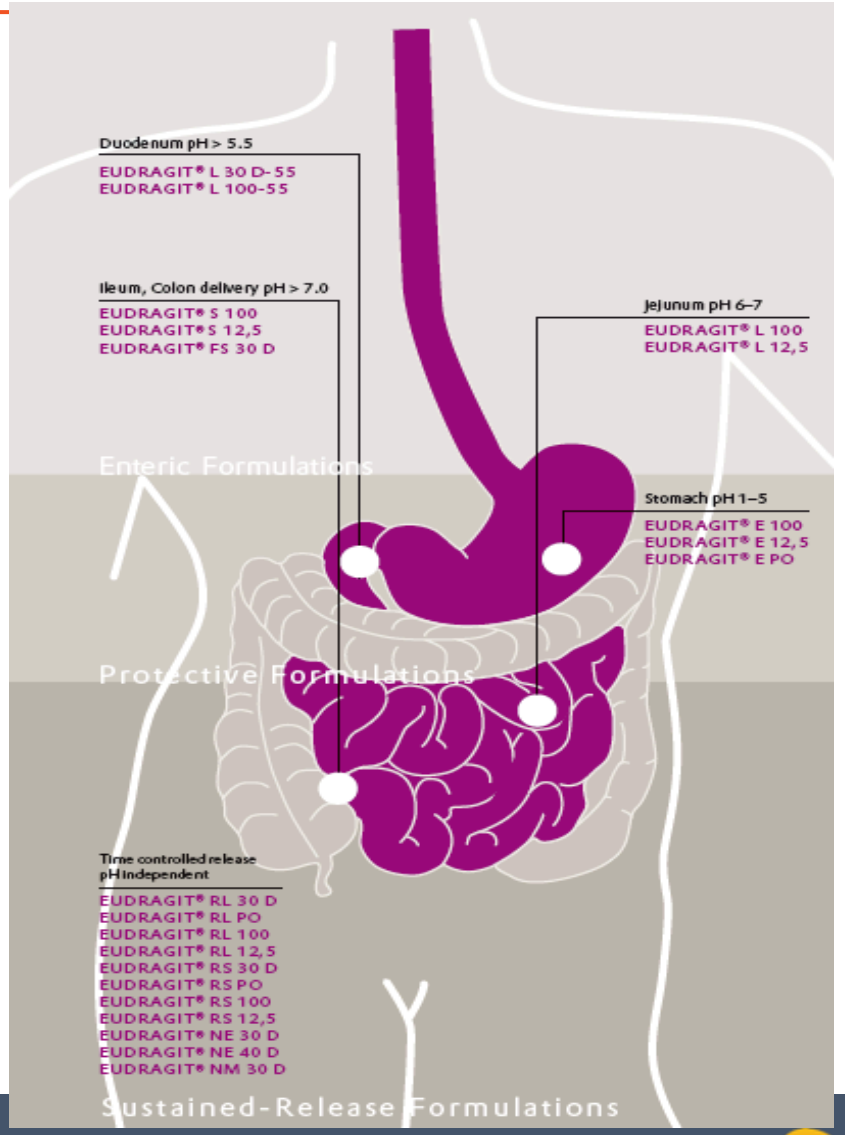

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SYN-004（ribaxamase)

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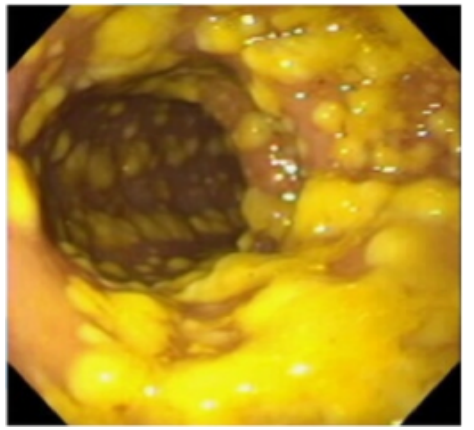
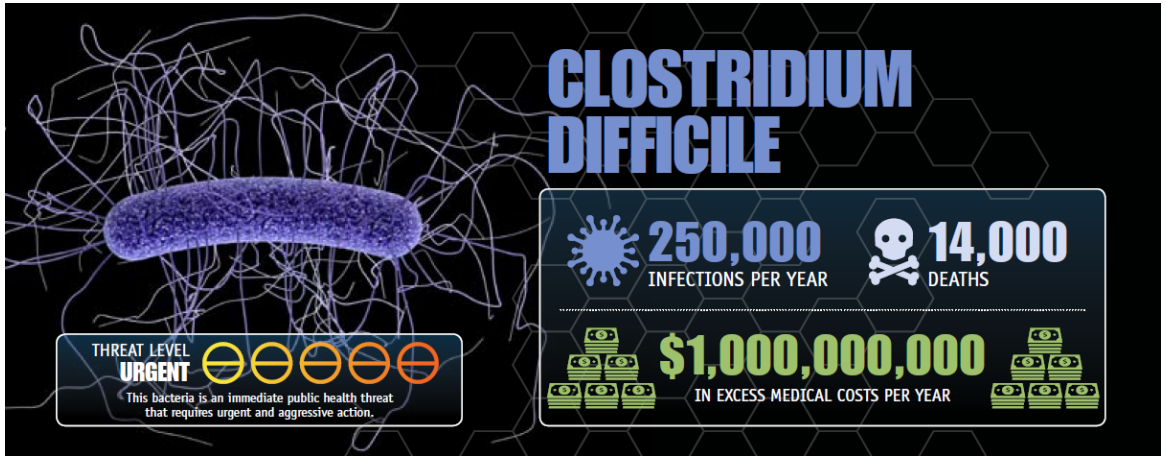
Clostridium difficile

C. difficile infection (CDI), *C. difficile*-associated disease (CDAD)

- Gram positive, spore forming bacteria
- Leading cause of antibiotic associated diarrhea
- Elaborates three primary toxins: Toxin A, Toxin B, and binary toxin
- Antibiotic resistance is a growing concern:
- *Diarrhea* *Pseudomembranous Colitis*
- *Toxic Megacolon* *Death*





Antibiotic Resistant Threats in the United States-2013, CDC

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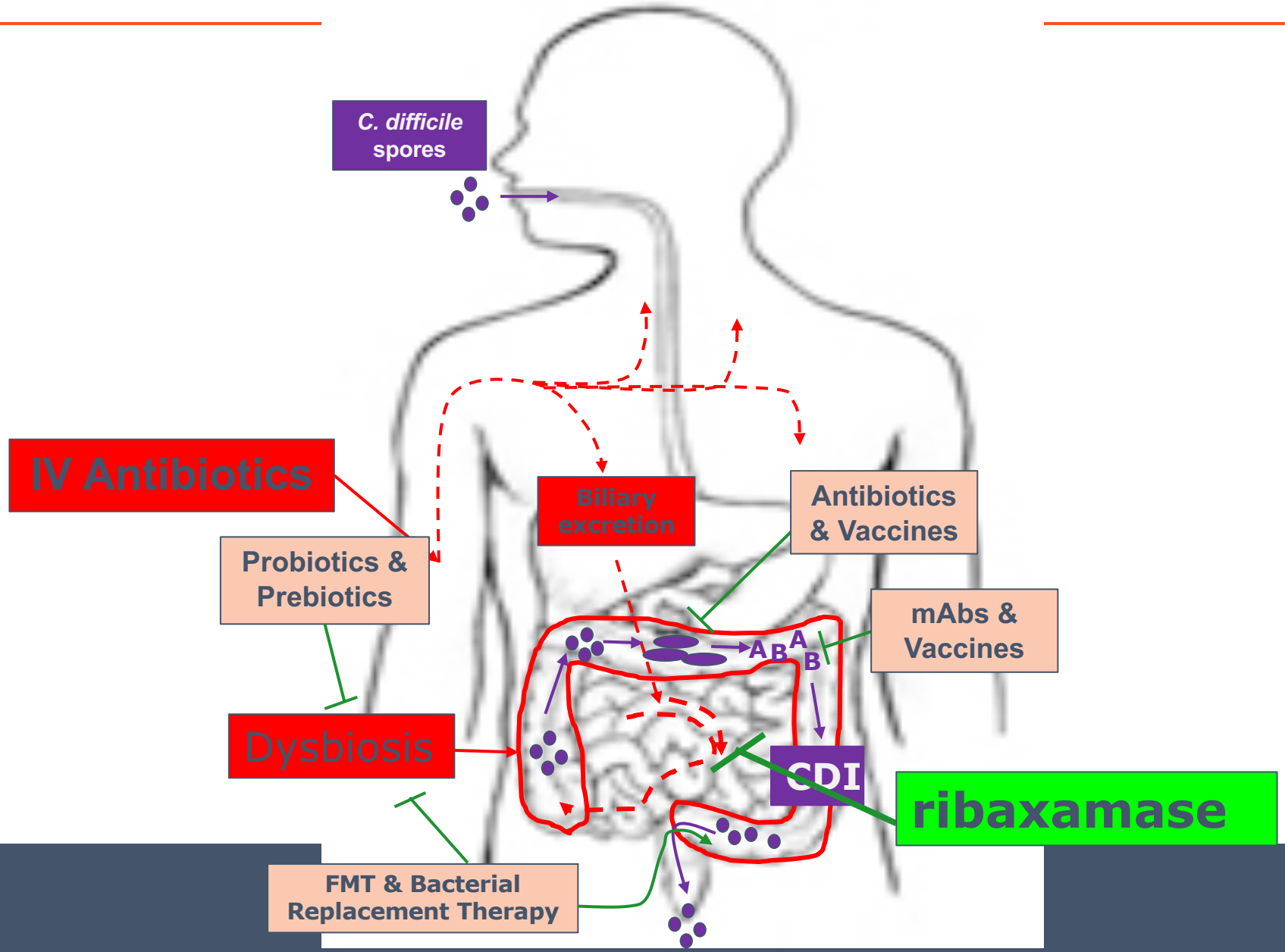
Disruption of the Gut Microbiome Can Lead to *C. difficile* Infection



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Ribaxamase (SYN-004)

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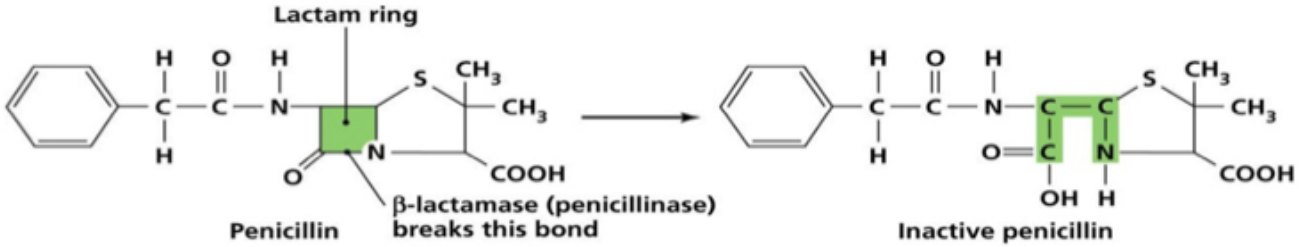
- Orally administered, β-lactamase enzyme (29 kDa) that is designed to degrade penicillins and cephalosporins
- Formulated for oral delivery with release at pH ≥ 5.5 (proximal small intestine)
- Expected to be administered during, and after administration of certain intravenous (IV) β-lactam-containing antibiotics, like ceftriaxone
- Intended to degrade the residual antibiotics that are excreted into the small intestine via the bile duct
- Designed to prevent disruption of the healthy gut microbiome and thus protect from opportunistic GI pathogens like *C. difficile*



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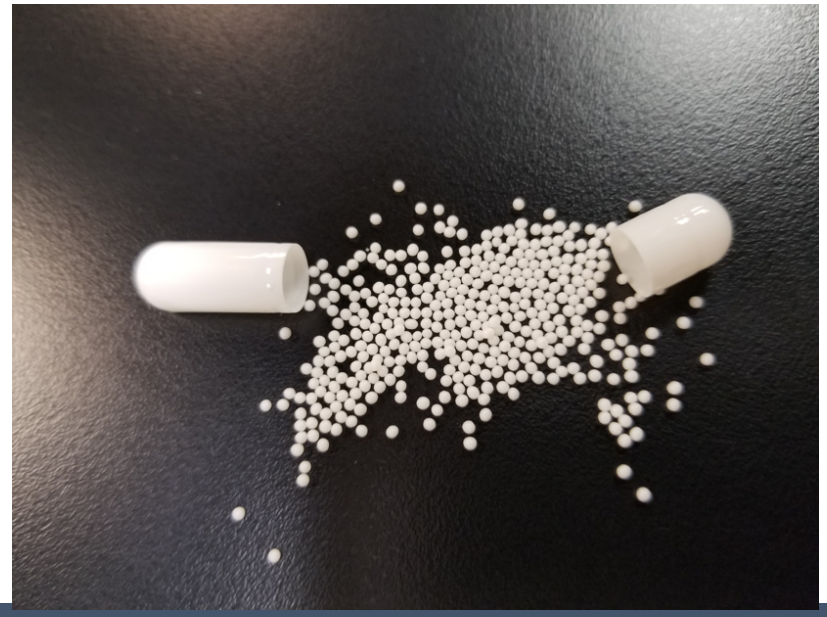
SYN-004 (ribaxamase) Formulation Development (1)

Desired Formulation Profile:

- Solid oral dosage form
- High dose of SYN-004
- Protection of active enzyme in gastric environment
- Multi-particulate
- Release in duodenum and proximal small intestine
- Simple, safe, well-known, readily available excipients
- Favorable stability for 2+ year shelf-life
- SYN-004 DS is purified from *E.coli*, supplied as liquid in phosphate buffer

Capsule containing:

- Multi-particulate pellets
- Enteric coated
- SYN-004 layered
- 75mg per capsule



SYN-004 (ribaxamase) Formulation Development (2)

SYN-004/Binder excipient screening/compatibility study using droplet film casting

Binder excipients:
Polyvinyl pyrrolidone (PVP) K-12
PVP K-25
PVP K-12 + glycerol
PVP K-12 + lactose
PVP K-12 + mannitol
PVP K-12 + sucrose
PVP K-12 + corn starch
PVP K-12 + PlasAcryl
Eudragit L30 D55 + glycerol
Eudragit NM 30D
HPC
HPMC

Additional excipients:
PEG 400
Triethyl citrate
Glycerol
1,2-propanediol
Glycerol monostearate
Polysorbate 80

Enteric coat:
Eudragit L30 D55

Binder excipients were mixed with several ratios of SYN-004 enzyme solution

In total, 123 free film castings were prepared and tested

Binder Excipient Screening; Compatibility with SYN-004

Casting of Free Films

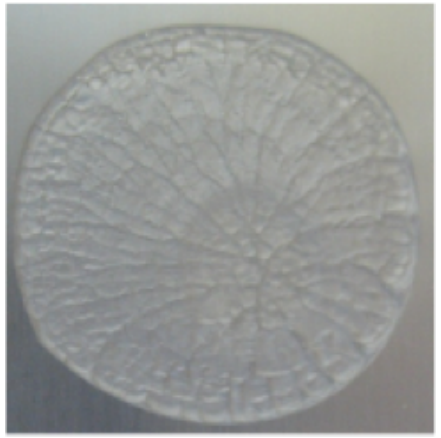
Polyvinyl pyrrolidone : SYN-004

The binding excipient **PVP K-12** was mixed with SYN-004 at ratios of:

1:1 (sample 22)
1:0.75 (23)
1:0.5 (24)
1:0.25 (25)
1:0.125 (26)

The films were dried to completion, stressed mechanically, and then photographed

Smoothness can predict success in fluid bed coating process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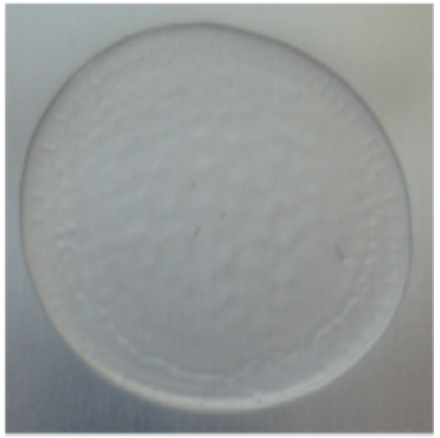
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25

26

Binder Excipient Screening; Compatibility with SYN-004

Casting of Free Films

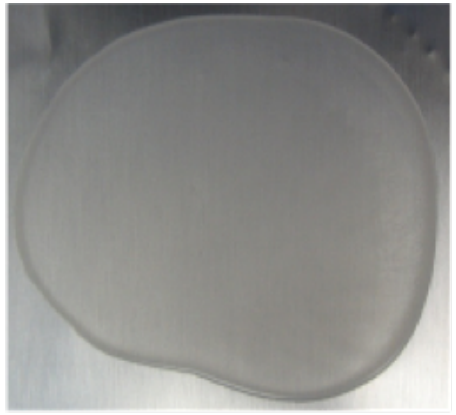
Hydroxypropylcellulose : SYN-004

The binding excipient **HPC** was mixed with SYN-004 at ratios of:

1:0.45 (sample 109)
1:0.40 (110)
1:0.35 (111)
1:0.30 (112)
1:0.25 (113)

The droplet films were dried to completion, stressed mechanically, and then photographed

Smoothness can predict success in fluid bed coating process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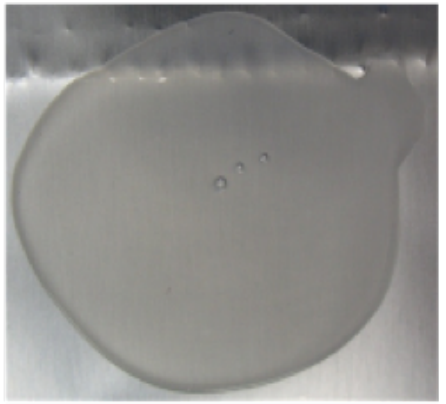
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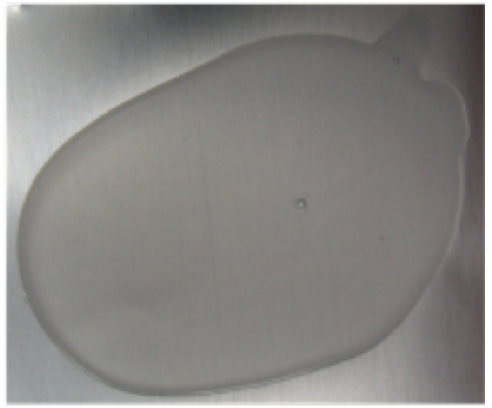
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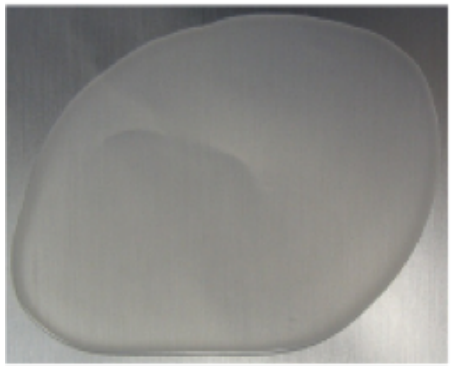
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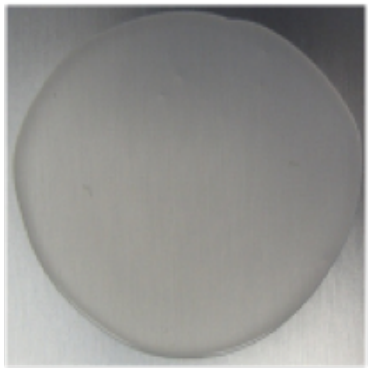
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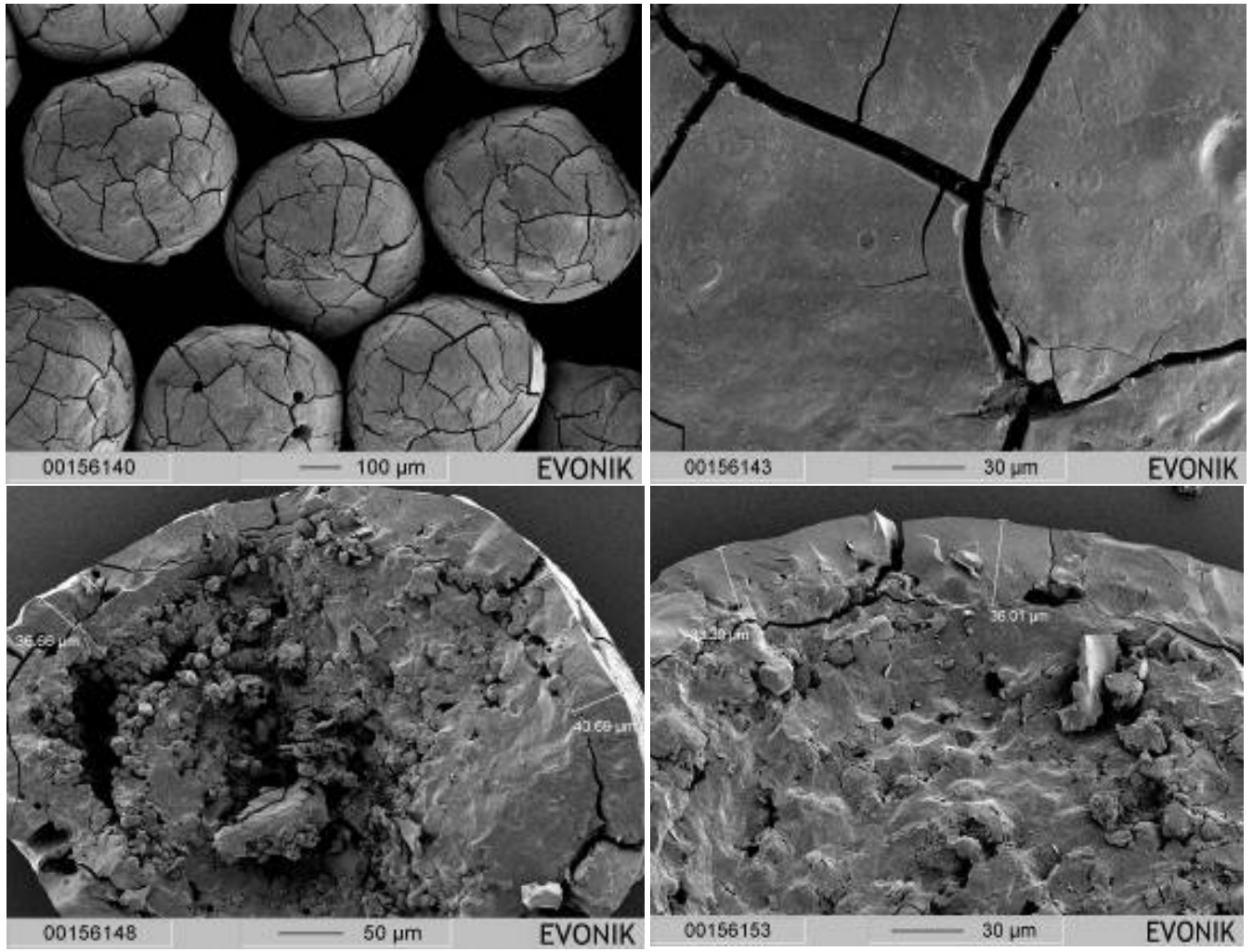
Drug Layering on Sucrose Cores by Fluidized Bed Spray Application

SYN-004 with binder **PVP K-12**
Coated onto sucrose cores in a fluidized bed
Examined by SEM

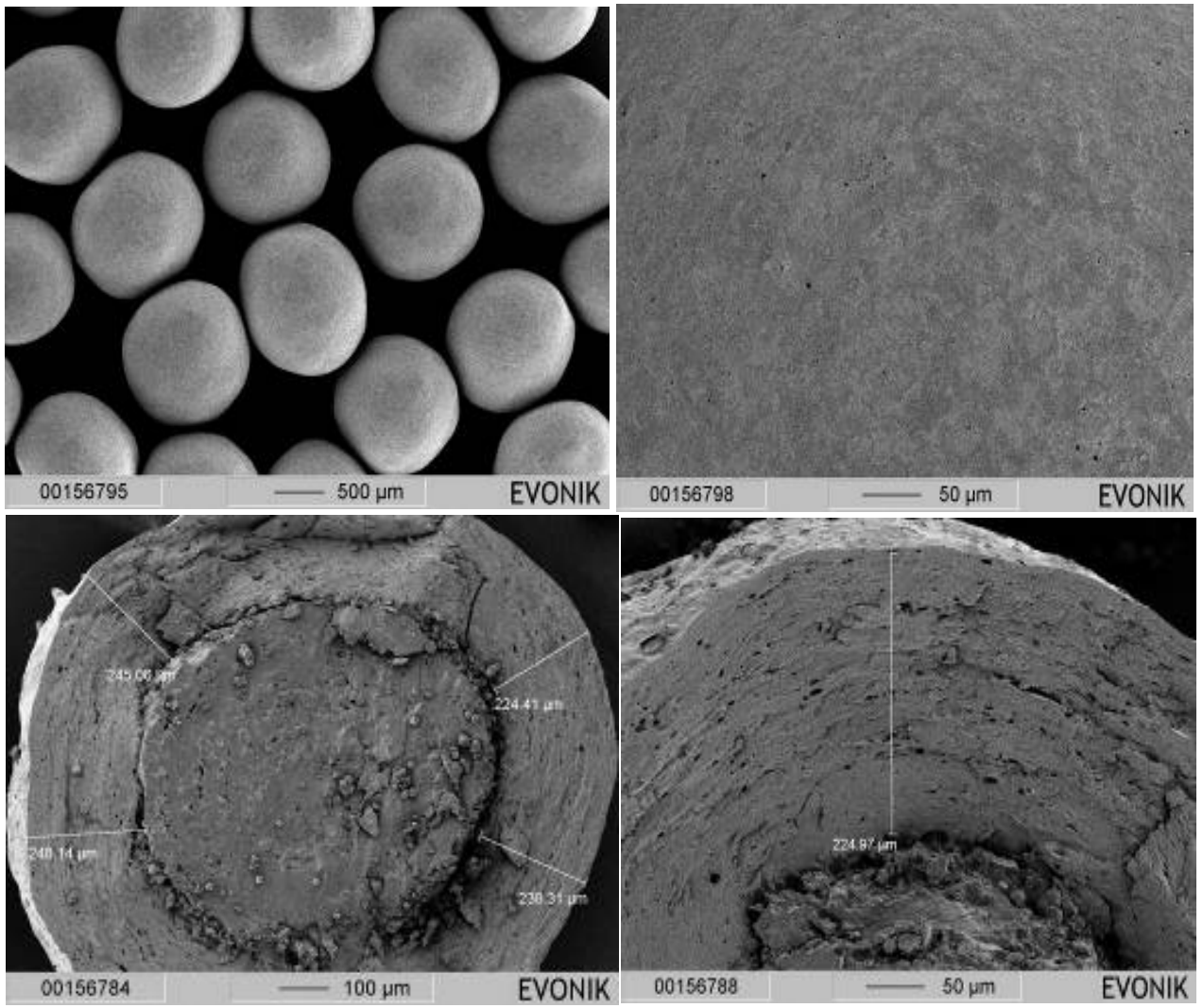
Result:
Deep surface cracks, poor matrix uniformity



Drug Layering on Sucrose Cores by Fluidized Bed Spray Application

SYN-004 with binder **HPC**
Coated onto sucrose cores in a fluidized bed
Examined by SEM

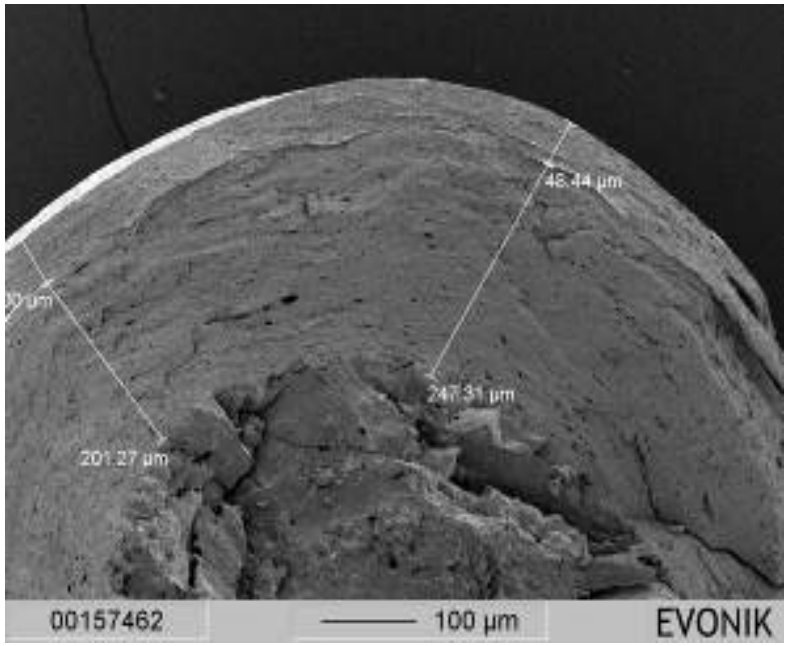
Result:
Smooth surfaces, uniform density

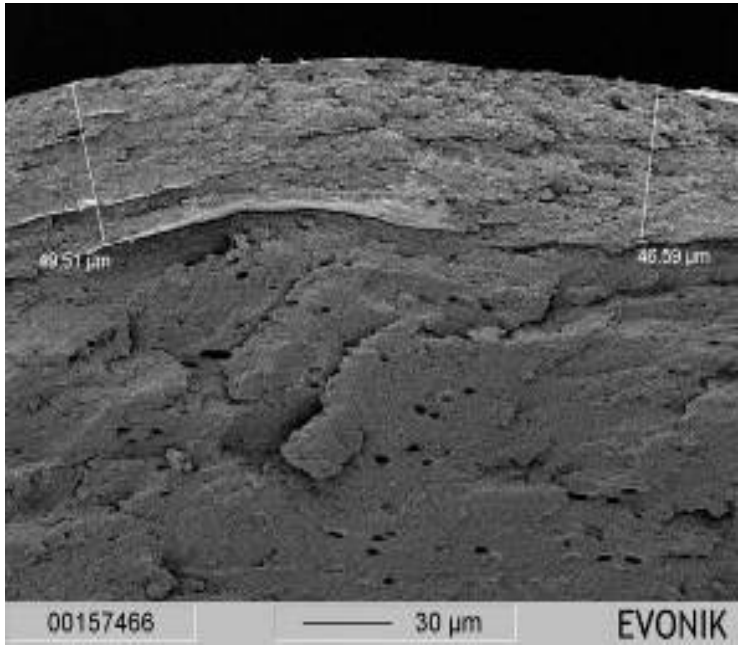


Enteric Coating of Drug Layered Cores by Fluidized Bed Spray Application

SYN-004 with binder **HPC**
Sprayed onto sucrose cores;
Cores sprayed with **Eudragit L30 D55**
Examined by SEM

Result:
Smooth surfaces, uniform density

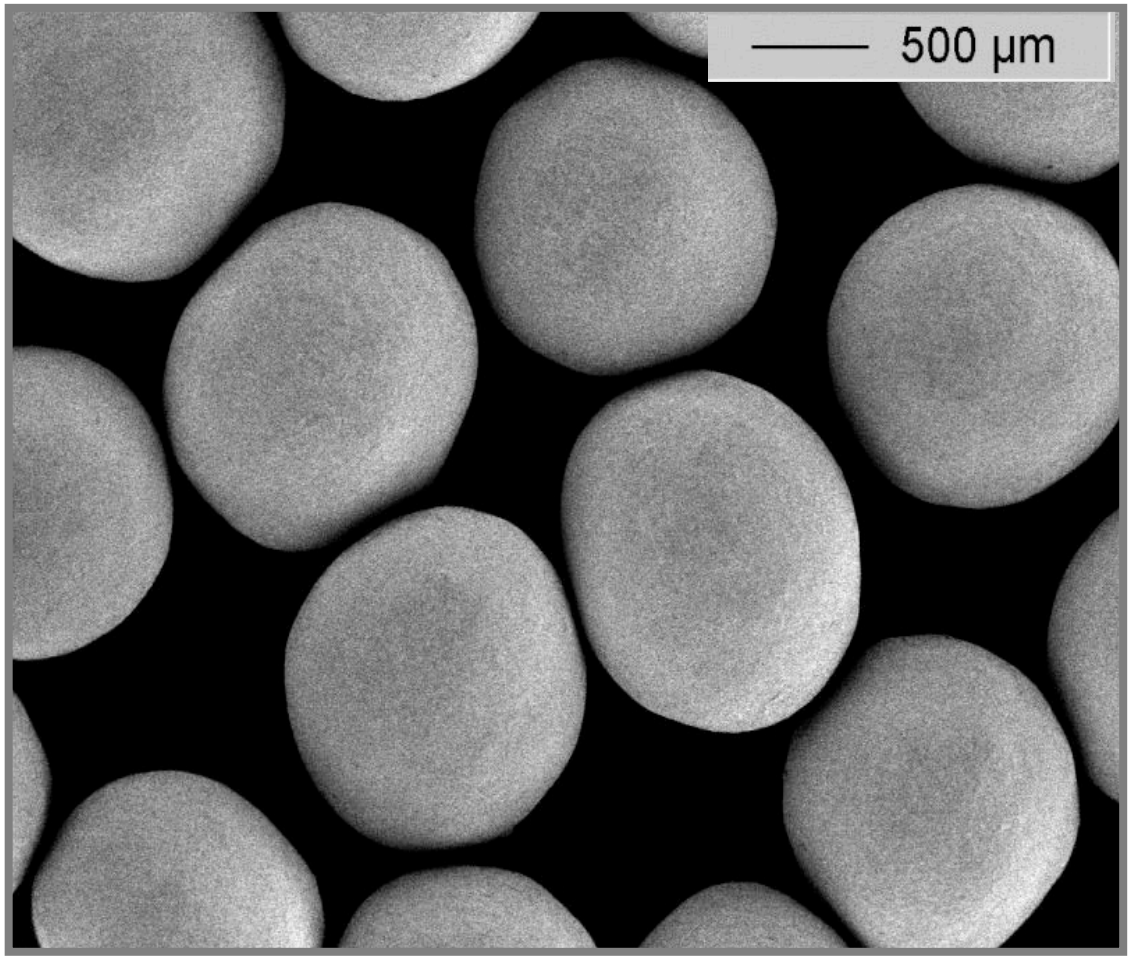




Drug layer 200-250 µm thick
Enteric coating 40-50 µm thick

SYN-004 (ribaxamase) Oral Formulation

Solid Dosage Format



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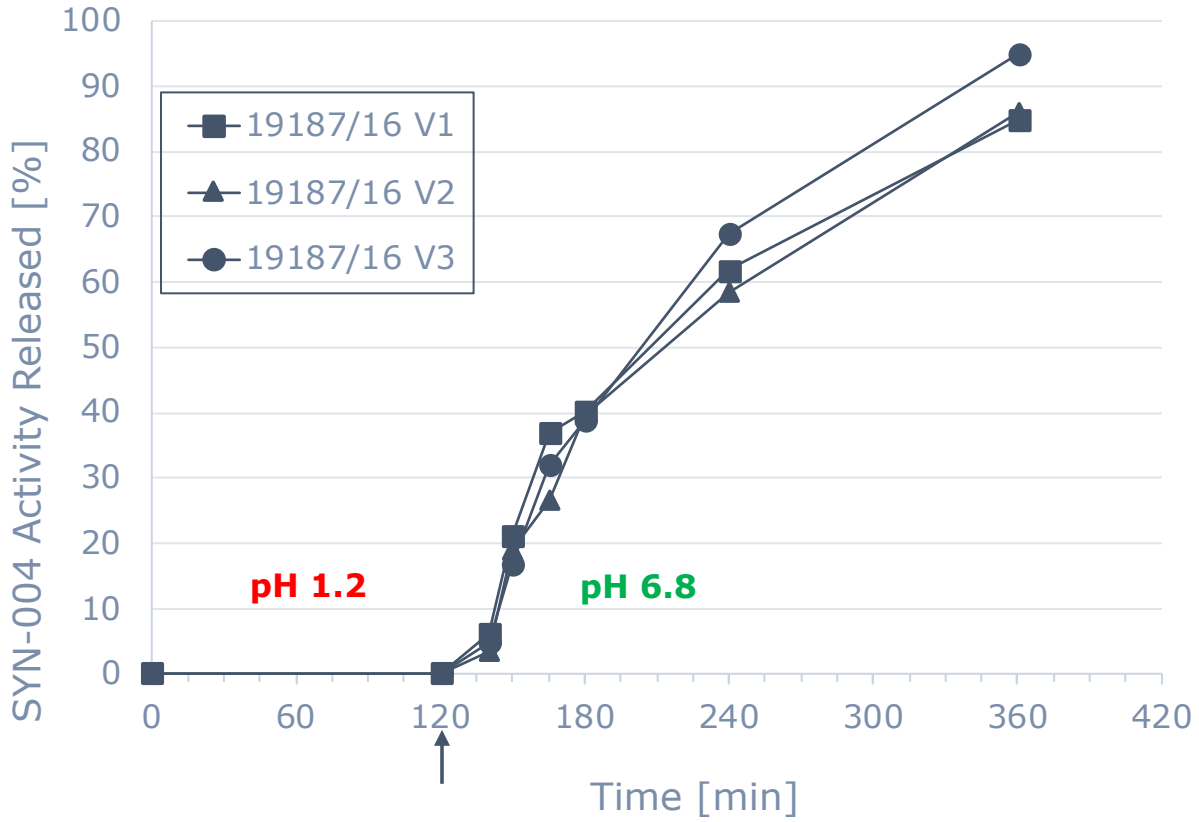
In Vitro Dissolution Profile of SYN-004 (ribaxamase)



- Early development Lot of SYN-004 enteric coated sucrose pellets
- USP Dissolution apparatus, type-2 (paddle)

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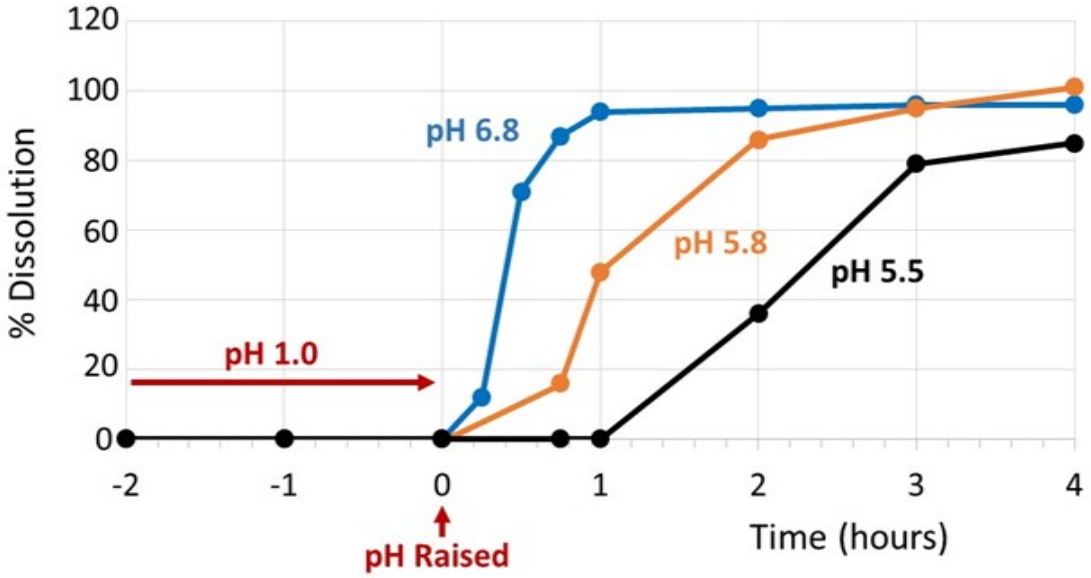
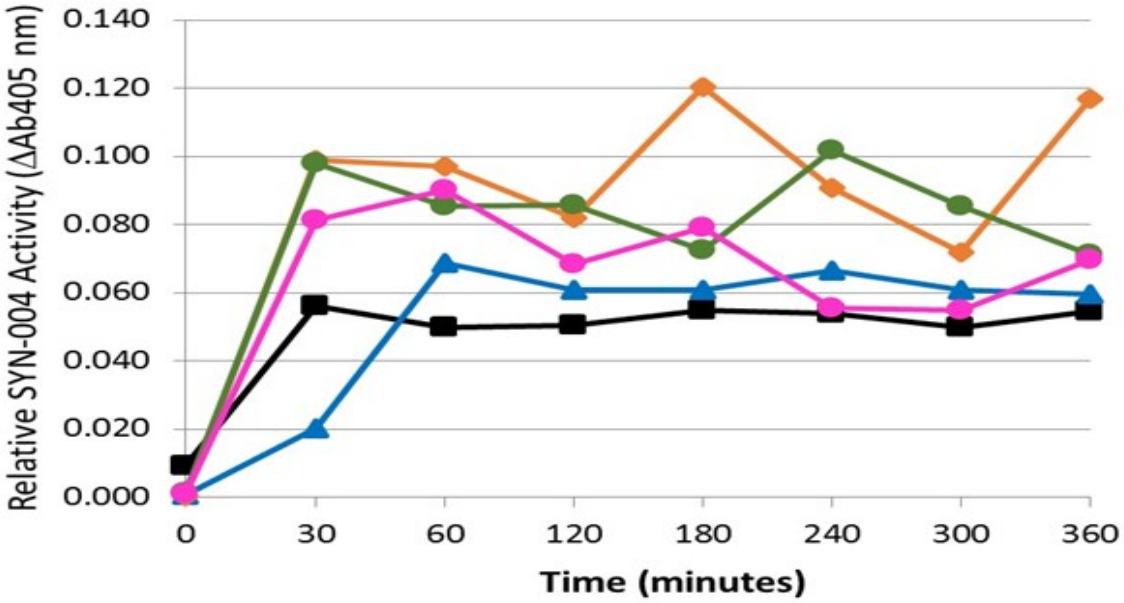
In Vitro Attributes of SYN-004 (ribaxamase) Oral Formulation

pH-Dependent Dissolution Profiles



Stability in Human Intestinal Chyme
(n=5 samples)



Enteric-coated ribaxamase pellets remain intact at low pH, and the released enzyme retains biological activity for at least 6 hours in human intestinal chyme

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SYN-004 (ribaxamase) Publications/Presentations

- A. Bristol, et al. **Formulation development of SYN-004 (ribaxamase) oral solid dosage form, a β-lactamase to prevent intravenous antibiotic-associated dysbiosis of the colon.** Int J Pharm 534:25–34 (2017).

- Kokai-Kun JF, et al. **The Oral β-Lactamase SYN-004 (ribaxamase) Degrades Ceftriaxone Excreted into the Intestine in Phase 2a Clinical Studies.** Antimicrobial Agents and Chemotherapy 61(3):e02197-16, (2017).

- Roberts T, et al. **Tolerability and Pharmacokinetics of SYN-004, an Orally Administered β-Lactamase for the Prevention of Clostridium difficile-Associated Disease and Antibiotic-Associated Diarrhea, in Two Phase 1 Studies.** Clin Drug Investig. 36(9):725-734, (2016).

- Kokai-Kun J, et al. **SYN-004 (ribaxamase) prevents New Onset Clostridium difficile Infection by Protecting the Integrity Gut Microbiome in a Phase 2b Study**. ID Week (2017).

- Kokai-Kun J, et al. **SYN-004 (ribaxamase), an Oral β-lactamase, Prevented Clostridium difficile Infection and Protected Patients from Colonization by Antimicrobial Resistant Pathogens by Preserving Gut Microbiome Diversity in a Phase 2b Clinical Trial**. DDW (2017).

SYN-010

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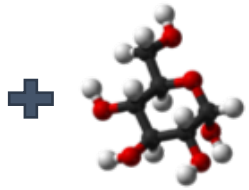
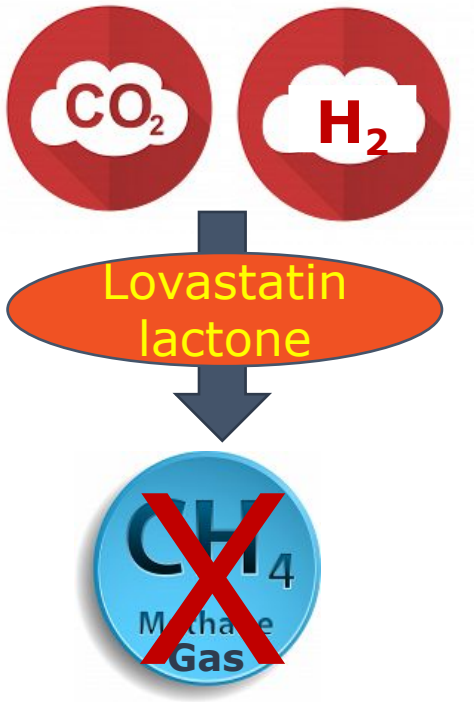
SYN-010: Proprietary Modified-Release Lovastatin Lactone

Designed to reduce methane production by *M. smithii* in the intestine



Carbohydrates



Bacteroides thetaiotaomicron is one of many bacterium that ferments carbohydrates in the gut which releases H_2 and CO_2

Methanobrevibacter smithii archea consumes hydrogen gas from *Bacteroides* and produces methane, which is lost from gut as "gas"

http://commons.wikimedia.org/wiki/File:Intestine_and_stomach_-_transparent_-_cut.png

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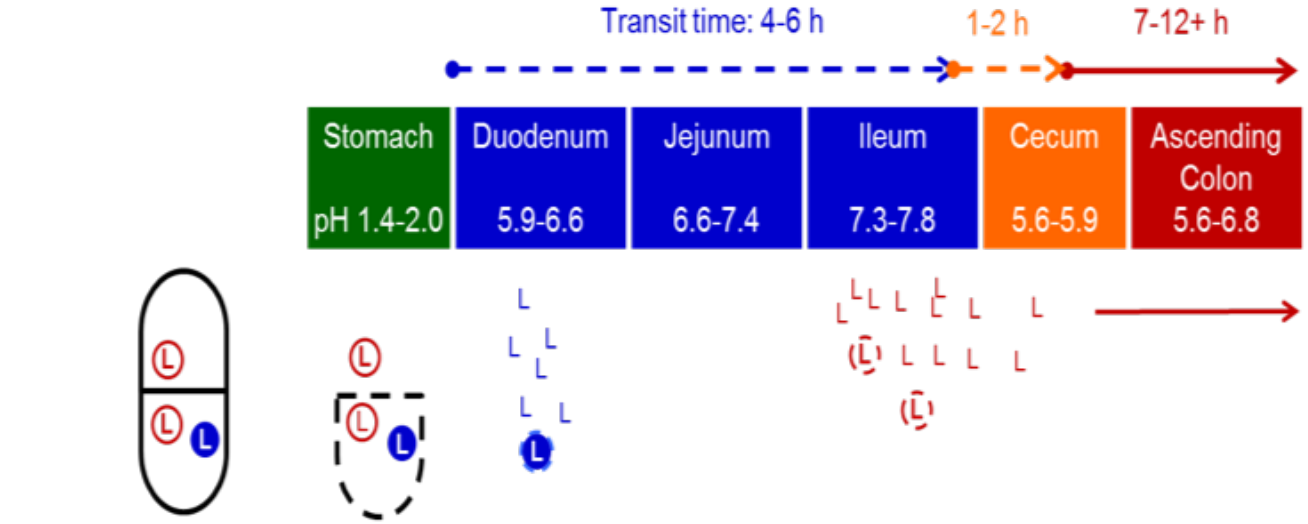
SYN-010 Formulation Design

Desired Formulation Profile:

- Solid oral dosage form
- Variable dose strength for Clin. Dev.
- Protection in gastric environment
- Dual pulse release: in duodenum and ileocecal junction
- Protect lovastatin lactone from conversion to the β-hydroxyacid (cholesterol lowering metabolite)
- Minimize or prevent systemic absorption
- Simple, safe, well-known, readily available excipients
- Favorable stability for 2+ year shelf-life



Lovastatin lactone API is supplied as dry powder

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Excipient Compatibility Testing

Excipient Compatibility, Binary Mixing Study

Excipients were mixed with lovastatin lactone, subjected to stress conditions for 1 week, tested for degradation of lovastatin lactone

% β-hydroxyacid, by HPLC

Batch #	Excipient:	Day 0	5ºC (7 days)	25ºC/60% RH (7 days)	40ºC/75% RH (7 days)
SB-18	N/A	0.03	0.00	0.00	0.00
SB-19	Pearlitol® 200SD	0.03	0.04	0.04	0.03
SB-20	Kollidon® VA64 Fine	0.04	0.05	0.04	0.05
SB-21	Kollidon® CL-F	0.05	0.05	0.05	0.06
SB-22	BHT	0.05	0.06	0.10	0.06
SB-23	Propyl Gallate	0.06	0.07	0.07	0.03
SB-24	Citric Acid Anhydrous	0.38	0.63	0.69	0.05
SB-25	AEROSIL® 200 Pharma	0.07	0.09	0.09	0.23
SB-26	EDTA	0.07	0.09	0.10	0.10
SB-27	PlasACRYL™ HTP20	0.08	0.12	0.12	0.22
SB-28	EUDRAGIT® L 30 D-55	0.11	0.19	0.19	0.27
SB-29	EUDRAGIT® FS 30 D	0.11	0.18	0.19	0.33

SYN-010 Compression Tableting Trials

Tablet cores:

Lovastatin lactone
Microcrystalline cellulose
Lactose monohydrate
Polyvinyl pyrrolidone VA
Crospovidone
Butylated hydroxyanisole
Propyl gallate
EDTA
Citric acid and maltodextrin
Silicon dioxide
Magnesium stearate
Pre-gelatinized starch
Mannitol
Calcium phosphate

Compressed tablet test parameters:

Weight
Hardness

Friability
Disintegration

Batch #	Mean Weight (mg)	Weight RSD (%)	Mean Hardness (KP)	Friability (% loss)	Disintegration (m : secs)
ANH-008	54.46	0.47	9.0	0.10	10:35
ANH-009	52.77	0.78	8.7	0.07	10:31
ANH-010	51.63	0.76	9.0	0.28	11:39

Excipients were blended with lovastatin lactone, compressed to 5mm round convex tablets, then tested

SYN-010 Tablet Coating Trials

Enteric coat:

Hydroxypropyl methylcellulose
Eudragit L30 D55
Eudragit FS30D
Glycerol monostearate
Triethyl citrate
Polysorbate 80
FD&C Blue #1, Aluminum lake (DR tablets only)

Core tablets were pan-coated, then tested

Coated tablet testing:

Weight gain on coating
Weight gain in acid uptake, blistering
Dissolution
Degradation to β-hydroxyacid metabolite

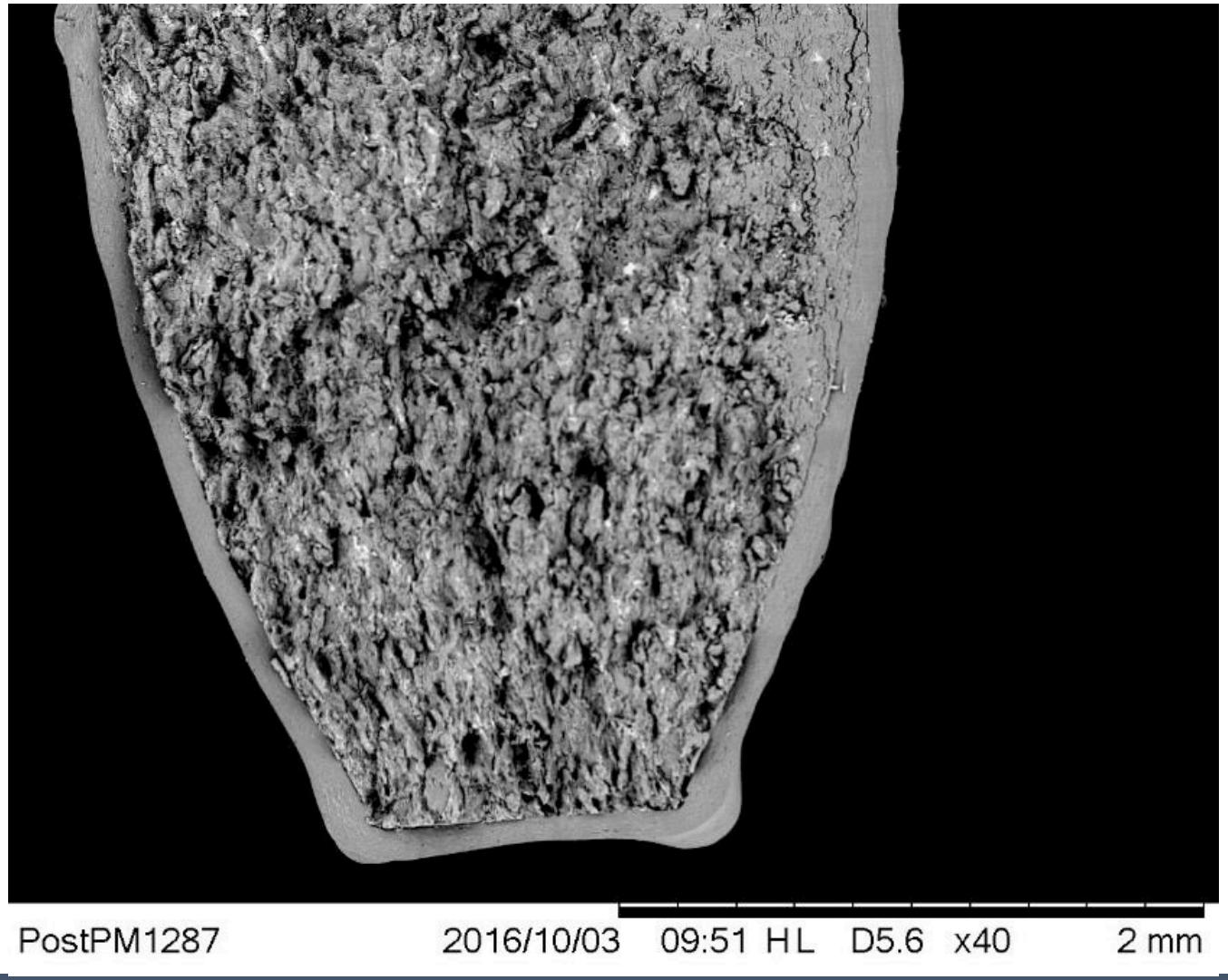
Batch #	Coat Loading (% w/w)	Weight of 6 Tablets Pre Acid Media (mg)	Weight of 6 Tablets Post Acid Media (mg)	Weight Gain (%)	Observations
ANH-055	6.28	329.5	350.9	6.5	Blistering of coat visible
ANH-051	8.60	333.7	350.9	5.2	Fully intact tablets
ANH-052	10.38	337.0	353.8	5.0	Fully intact tablets
ANH-053	14.61	355.4	367.3	3.3	Fully intact tablets

SYN-010 Tablet Formulation

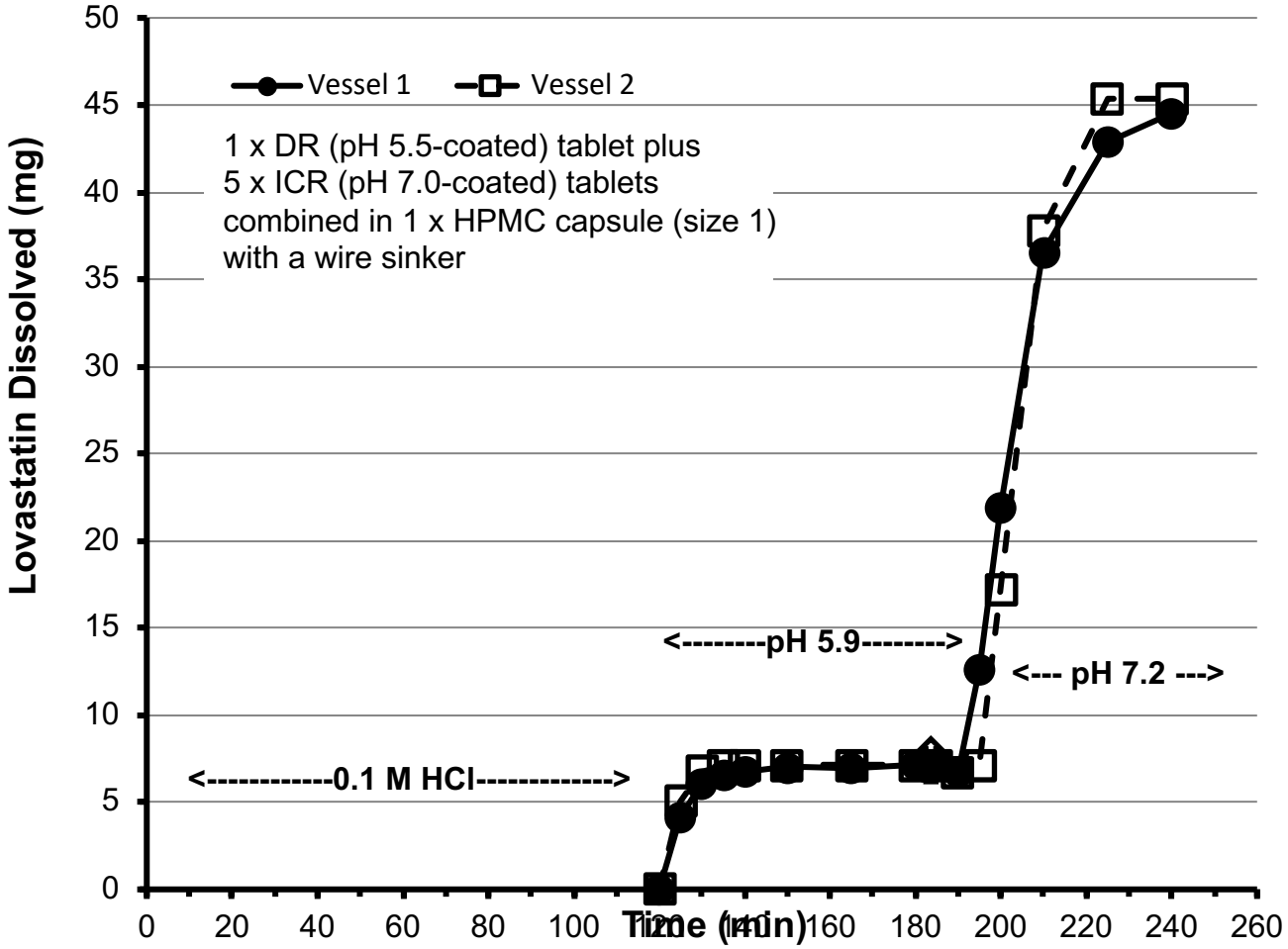
Solid Dosage Form

SYN-010 lovastatin lactone compressed with dry excipients;
Uncoated cores sprayed with **Eudragit L30 D55** or **Eudragit FS30D;**
Examined by SEM

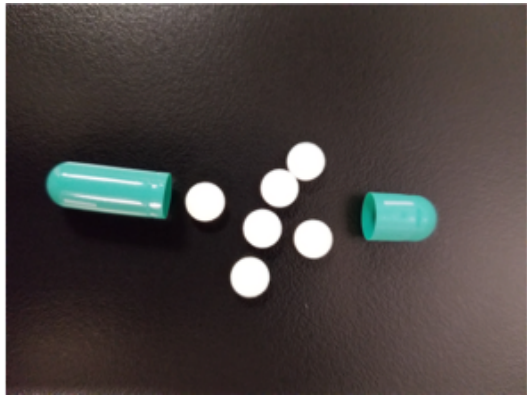
Result:
Tight, uniform matrix density
Smooth coated surface



In Vitro Dissolution Profile of SYN-010



- Early development Lot of SYN-010 enteric coated tablets, encapsulated
- USP Dissolution apparatus, type-2 (paddle)



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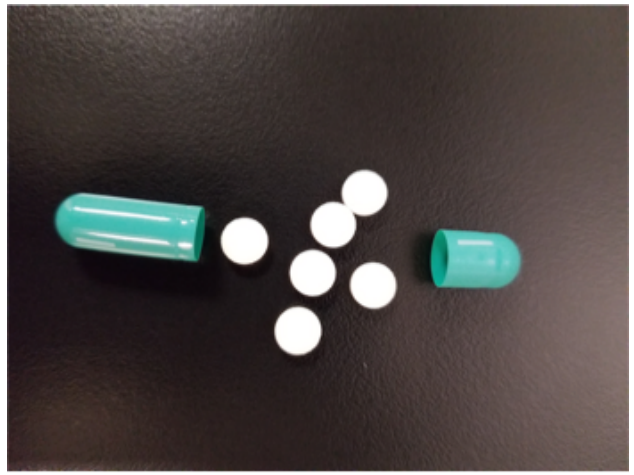
SYN-010 Oral Dosage Format

Desired Formulation Profile:

- Solid oral dosage form
- Variable dose strength for Clin. Dev.
- Protection in gastric environment
- Dual pulse release: in duodenum and ileocecal junction
- Protect lovastatin lactone from conversion of the to β-hydroxyacid (cholesterol lowering metabolite)
- Minimize or prevent systemic absorption
- Simple, safe, well-known, readily available excipients
- Favorable stability for 2+ year shelf-life

Capsule containing:

- 7mg tablet coated for release in duodenum (L30 D55)
- 7mg tablet coated for release in ileocecal junction (FS30D)
- Targeted API delivery can be dosed up or down in 7mg units



SYN-010 Publications/Presentations

- S. Hubert, et al. **Development of a Modified-Release Formulation of Lovastatin Targeted to Intestinal Methanogens Implicated in Irritable Bowel Syndrome With Constipation.** J. Pharm Sci; 107:662-671 (2018).

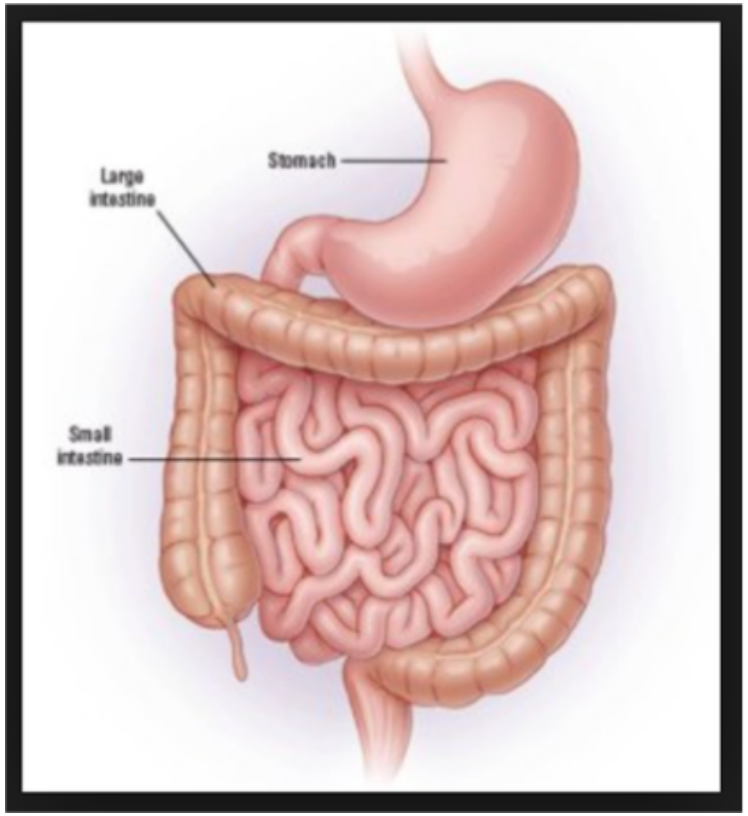
- V. Wacher, et al. **Pharmacokinetics of SYN-010 modified-release lovastatin lactone in healthy volunteers: methane-reducing drug levels are delivered to the colon at doses that alleviated symptoms of irritable bowel syndrome with constipation (IBS-C) in Phase 2a clinical trials.** JDDW (2017).

- V. Wacher, et al. **SYN-010, a Proprietary Modified-Release Formulation of Lovastatin Lactone, Lowered Breath Methane and Improved Stool Frequency in Patients with IBS-C.** APDW (2016).

- K. Gottlieb, et al. **Review article: Inhibition of methanogenic archaea by statins as a targeted management strategy for constipation and related disorders.** Aliment Pharmacol Ther; 43:197–212 (2016).

Conclusions

- Oral drug products for localized gut microbiome activity are an increasing new class of drug products
- Need for targeted intestinal delivery is important
- Whether a biological or small molecule, formulation is critical:
  - Protection from stomach
  - Desired release profile
  - Stability in intestinal environment
  - Limited absorption or non-absorbed
  - Co-localization to the drug's target molecule or organism
- Considerations include excipient selection, compatibility screening studies, optimization studies, analytical methodology



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Questions?

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