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Research Paper

Formulation development of SYN-004 (ribaxamase) oral solid dosage form, a β-lactamase to prevent intravenous antibiotic-associated dysbiosis of the colon

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ABSTRACT

SYN-004 (ribaxamase) delayed release drug product is a multi-particulate, hard capsule for oral delivery of a recombinant β-lactamase enzyme designed to degrade β-lactam antibiotics administered intravenously, and thus prevent colon dysbiosis. Here we describe the development of the SYN-004 enteric coated pellet formulation, which has been tested in multiple clinical trials. Since the SYN-004 drug substance is a buffered liquid, several binder excipients in different ratios were tested to facilitate binding of SYN-004 to sugar spheres. The binding systems were evaluated by droplet pre-evaluation and film casting tests. The most promising formulations were produced in small scale fluidized bed application runs and analyzed by dissolution tests and complementary analytical assays. Hydroxypropyl cellulose was selected as the preferred SYN-004 binding excipient. The formulation included a second, outer coat containing the enteric EUDRAGIT® L 30 D-55 polymer-based formulation to achieve gastric protection, and rapid SYN-004 release in the intestinal tract, when the pH rises above 5.5. Additional formulation improvements resulted in an increase in the SYN-004 load compared to a predecessor oral enzyme formulation (Ipsat P1A). Thus, a novel formulation and process for an orally administered enzyme was developed and used to manufacture drug product for clinical trials.

1. Introduction

There is a well-established association between the use of antibiotics, in particular those belonging to the β-lactam class such as ceftriaxone, and colon dysbiosis (a disruption of the balance of microorganisms of the colon) (Bartlett, 2002). Of particular concern is the development of *Clostridium difficile*-associated diarrhea (CDAD) and antibiotic-associated diarrhea (AAD) (Bartlett, 2002; Carroll and Bartlett, 2011; Drekonja et al., 2011; Larcombe et al., 2016; Zycinska et al., 2016). Thus, there is a need for new medical interventions that reduce the risk of developing these secondary infections that are associated with the use of intravenously (IV) administered β-lactam antibiotics, of which a substantial portion are excreted into the intestine following IV administration (Maudgal et al., 1982; Karachalios and Charalabopoulos, 2002).

SYN-004 (ribaxamase) is a recombinant β-lactamase, formulated as an oral delayed release capsule designed to be administered to patients receiving SYN-004 susceptible IV β-lactam antibiotics. Upon release of SYN-004 drug substance (DS) from the drug product (DP) into the proximal small intestine, SYN-004 degrades susceptible IV administered β-lactam antibiotics that are excreted into the gastrointestinal (GI) tract. It is believed that SYN-004 will prevent undesirable effects including AAD, CDAD, and the development of antibiotic resistant organisms and other secondary infections in the gut related to antibiotic administration. SYN-004 has been tested in multiple clinical trials (Roberts et al., 2016; Kokai-Kun et al., 2017), and is not systemically available when delivered orally in the delayed release formulation (Kokai-Kun et al., 2016). The mechanism of action of SYN-004 is the inactivation of susceptible β-lactam antibiotics by hydrolysis of the amide bond of the β-lactam ring (Deshpande et al., 2004). The active SYN-004 enzyme is manufactured by recombinant DNA technology by fermentation using *E. coli* [reviewed by Tripathi, 2016], and is purified

Abbreviations: AAD, antibiotic-associated diarrhea; CDAD, *Clostridium difficile* associated diarrhea; DP, drug product; DS, drug substance; GMS, glycerol monostearate; HPC, hydroxypropyl cellulose; HPMC, hydroxypropyl methylcellulose; IIG, inactive ingredient guide; PS80, polysorbate-80; RSD, relative standard deviation; SEM, scanning electron microscopy; TEC, triethyl citrate

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to near homogeneity by a single column chromatography step. The final bulk DS is formulated in potassium phosphate buffer at high protein concentration.

A predecessor oral enzyme product, called Ipsat P1A, was formulated as a multi-particulate pellet containing capsule and tested in multiple clinical trials, where it was shown to be safe and well tolerated. In healthy volunteers, oral P1A prevented IV ampicillin-induced alterations in intestinal microflora and the emergence of antibiotic resistance (Tarkkanen et al., 2009). The goal for the P1A drug delivery strategy was to achieve continuous entry of small intact enteric coated sugar pellets into the duodenum through the pylorus. The previous P1A formulation achieved only approximately 6% loading of P1A and used EUDRAGIT® L 30 D-55 as both the binding excipient and as the enteric coat (Tarkkanen et al., 2009). A similar multi-particulate delivery strategy was maintained for the current SYN-004 DP formulation, although several improvements to the formulation were desired, including: 1) reducing the amount of EUDRAGIT® L 30 D-55 by replacing the binding excipient with a common alternative binder, 2) increasing the level of SYN-004 enzyme in the final coated pellet to greater than 10% SYN-004, 3) ensuring a coated pellet formulation with enteric protection of the SYN-004 enzyme during two (2) hours in 0.1 hydrochloric acid followed by rapid release of the enzyme in pH 6.8 buffer, and 4) ensuring a smooth, crack-free surface with low sticking properties. A higher drug loading on the pellets was desired to facilitate a higher dose of active enzyme and potentially smaller capsules. Further, it was desirable to replace the EUDRAGIT® L 30 D-55 binding excipient with an alternative binding excipient, to keep the amount of EUDRAGIT® L 30 D-55 exposure in the intestine below levels published in the Inactive Ingredient Guide (IIG) Database (Inactive Ingredients database, 2017) [http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm]. This article describes the development of the improved SYN-004 DP formulation used in nonclinical and early phase clinical trials.

2. Materials and methods

The purified SYN-004 bulk enzyme used in these studies was provided by a contract manufacturing partner as a liquid of approximately 100 mg/mL protein in a solution of 50 mM potassium phosphate pH 7.2. The excipients and other materials were purchased commercially as follows: sucrose sugar spheres (Pharm-a-spheres neutral 600–710 μm from Hanns G. Werner GmbH & Co. KG, Tornesch, Germany), polysorbate-80 (Merck KGaA, Darmstadt, Germany), glycerin (Merck KGaA, Darmstadt, Germany), lactose (Pharmatose® 450 M, DFE Pharma, Goch, Germany), polyvinyl pyrrolidone (PVP) (Kollidon® K12 and K25, BASF, Ludwigshafen, Germany), hydroxypropyl cellulose (HPC) (Klucel™ EF Pharm, Ashland Specialty Ingredients G.P., USA), Triethyl Citrate (TEC) (CITROFOL® AI, Merck KGaA, Darmstadt, Germany), low viscosity hydroxypropyl methylcellulose (HPMC) (Sheffield Biosciences, USA), Coni-Snap® hard gelatin capsules size #0 white/white (CAPSUGEL Colmar, France), methacrylic acid – ethyl acrylate copolymer dispersion 30% (EUDRAGIT® NM 30 D, Evonik Nutrition & Care GmbH, Darmstadt, Germany), methacrylic acid – ethyl acrylate copolymer (1:1) dispersion 30% (EUDRAGIT® L 30 D-55, Evonik Nutrition & Care GmbH, Darmstadt, Germany), glycerol monostearate 40–55% (GMS) (Imwitor® 900K, Sasol, Witten, Germany). The CENTA substrate used to determine SYN-004 β-lactamase activity was from Merck Calbiochem (CAS 9073-60-3). Other laboratory equipment used, such as stirrers, mixers, and balances were standard equipment, and manufacturing equipment used are described as presented below.

2.1. Binding excipient screening by casting of free films

Alternative binding excipients to replace EUDRAGIT® L 30 D-55 as the API binding agent were screened by droplet pre-evaluation and film application testing. First, a suspension of SYN-004 was mixed with various binder excipients and at various ratios [Polyvinylpyrrolidone (PVP) K-12, PVP K-25, Hydroxypropylcellulose (HPC), Hydroxypropyl methylcellulose (HPMC)]. Next, a film application plate (Erichsen Coatmaster 500, Erichsen GmbH, Germany) pre-heated to 35–40 °C was covered with aluminum foil. Approximately 0.5 mL of each solution was added to the foil as droplets, then a film was created by drying the solution for 10–24 h at room temperature (20–25 °C). Once dried, the film was qualitatively evaluated by macroscopic optical visualization. Furthermore, the dried casted films were twisted slightly in order to evaluate the mechanical stability. This test was performed to rate the film properties on a matrix, which was used as a first indication to support the selection of a suitable binder excipient and binder:API ratio. Therefore, in some cases, a plasticizer, a glidant, or other common suitable excipient was added to increase the flexibility and stability of the film.

2.2. Fluid bed coating of sucrose sugar spheres

The manufacture of SYN-004 delayed-release capsule was a three stage sequential process comprised of: 1) SYN-004 DS layering onto sugar spheres by spray application, 2) enteric coating with EUDRAGIT® L 30 D-55 by spray application, and 3) encapsulation of pellets into size 0 hard capsules. Encapsulation for analytical testing was manual; encapsulation for the clinical batch was conducted on a Labby capsule filler (MG2, Italy). During the formulation development, two different scales of fluid bed equipment were used for each of the spray application steps. The early drug layering and enteric coating trials were conducted using the Oystar Huttlin Mycrolab Fluid Bed Dryer (Huttlin GmbH, Germany) with bottom spray and microclimate technology (up to 80 g starting spheres). The spray gun used on the Oystar Huttlin equipment was a Huttlin 3-component nozzle with a 0.6 mm nozzle bore. Larger scale batches were produced using the GPCG-3.1 with bottom spray (Glatt GmbH, Germany) (up to 930 g starting spheres). The spray gun used on the GPCG-3.1 equipment was a Schlick 970/0-S3 (Dusen-Schlick GmbH, Germany) with a 0.8 mm nozzle bore for the drug layering and 1.2 mm nozzle bore for the enteric polymer coating.

2.3. Pellet morphology analysis

Scanning Electron Microscopy (SEM) was performed to study the cross-sectional and surface area morphology of the polymeric films on coated sugar spheres. As a preparation step, the samples were broken and sputter-coated with gold for conductance. Examination of the samples was carried out using a Jeol JSM-840 A instrument (Jeol GmbH, Germany) operating at an accelerating voltage of 5 kV.

2.4. Analytical methodology

Dissolution testing based on the United States Pharmacopeia (USP) methodology (USP<711> for Apparatus 2) for delayed release dosage forms was employed. The method used dissolution conditions of 2 h in 0.1N HCl pH 1.2, followed by 4 h in phosphate buffer pH 6.8. Samples were collected after 2 h in acid, then at 15, 30, 45, 60, 120, and 240 min at pH 6.8. The samples were tested for β-lactamase activity using the CENTA substrate (Jones et al., 1982) in a microtiter plate based assay. The assay involves pipetting 50 μL of standards (0.05–0.65 mg/L range), control, blank, or dilutions of unknown samples into microtiter plate wells, then equilibrating the plate for 20 min at 25 °C. Then 200 μL of CENTA solution (1 mM working solution, also equilibrated for 20 min at 25 °C) is pipetted into wells A1-A8 using a multi-channel pipet, and the row is read immediately using a plate reader with wavelength set at 405 nm, measuring every 8 s to collect 8 data points. Additional samples were similarly analyzed in turn, in rows B, C, and so on. The concentrations of unknown samples were interpolated from the standard curve, and the results were plotted as β-lactamase activity versus sampling time of the dissolution analysis.

Table 1
Results of film coating SYN-004 with excipients.

Ratio of Excipient/SYN-004 (w/w)			Optical Evaluation Result
Excipient (Sample ID)	Excipient	SYN-004	
PVP K-25.	1	1–0.125	Cracks in film
PVP K-12 (22)	1	1	Cracks in film
PVP K-12 (23)	1	0.75	Cracks on frame of film
PVP K-12 (24)	1	0.5	No cracks, but uneven surface
PVP K-12 (25)	1	0.25	No cracks, smooth film
PVP K-12 (26)	1	0.125	No cracks, smooth film
HPMC	1	1–0.125	Cracks in film
Eudragit L30 D55	1	5–0.125	Cracks in film
PEG 400	1	1–3	Cracks in film
TEC	1	1–3	Cracks in film
Glycerol	1	1–3	Cracks in film
1,2-propanediol	1	1–3	Cracks in film
EUDRAGIT® L 30 D-55 + 20% Glycerol	1	3–0.25	Cracks in film
EUDRAGIT® L 30 D-55 + 20% Glycerol	1	0.125	No cracks, smooth film
PVP K12 + 20% Glycerol	1	2–0.4	Cracks in film, uneven surface
PVP K12 + Pharmatose 450M	1	1	No cracks, smooth film
PVP K12 + Pharmatose 450M	1	3–2	Cracks in film
PVP K12 + Mannit Pearlitol	1	1	Cracks in film
PVP K12 + Mannit Pearlitol	1	3–2	No cracks, smooth film
PVP K12 + sucrose	1	1	No cracks, smooth film
PVP K12 + sucrose	1	3–2	Cracks in film
PVP K12 + corn starch	1	3–1	Cracks in film
PVP K12 + lactose	1	1–0.2	Cracks in film
EUDRAGIT® L 30 D-55 + NaOH neutralization	1	3–1	Cracks in film
PVP K12 + PlasACRYL HTP20	1	1–0.2	Cracks in film
HPC Klucel EF (109)	1	0.45	No cracks, smooth film
HPC Klucel EF (110)	1	0.40	No cracks, smooth film
HPC Klucel EF (111)	1	0.35	No cracks, smooth film
HPC Klucel EF (112)	1	0.30	No cracks, smooth film
HPC Klucel EF (113)	1	0.25	No cracks, smooth film
HPC Klucel EF	1	0.75	No cracks, smooth film
HPC Klucel EF	1	4–1	Fine cracks in film

Triplicate samples from the two enteric coated sugar sphere batches (19187/16 and 19187/20) were tested and plotted individually in each graph (designated V1–V3). Water content (USP⟨921⟩) of drug layered and enteric coated pellets were conducted under contract at PHAST GmbH (Homburg, Germany). The analytical methods were validated prior to manufacture of GMP batches for clinical trials; however, the methods were not yet validated for testing the development batches described here. The enzyme assay using CENTA substrate was successfully validated for: specificity (endpoint OD values were significantly higher than blanks), linearity ($r^2 = 1.00$), accuracy (analyte recovery between 90 and 110%), range (0.05–0.65 mg/L), repeatability (RSD < 10%), intermediate precision (RSD < 10%), and stability of solutions (1 day at ambient temperature, 6 days at −20 °C).

3. Results

3.1. Excipient screening by casting of free films

The method employed to study the physical compatibility between excipients and SYN-004 protein in preparation for solid oral dosage production was a droplet-screening panel. The chemical compatibility was not the focus of the droplet-screening study. The main interests were the physical compatibility parameters of film forming ability and API binding capacity. The resulting dried droplets were macroscopically evaluated with the purpose to determine lead formulations for the first trials of drug layering on sucrose pellets in fluidized bed coating equipment. Thus, common parameters for chemical incompatibility such as heat and humidity were excluded at the stage of casting of free films. The chemical stability of the final drug layering formulation was evaluated during the fluid bed layering process development as discussed below.

Initially, Polyvinylpyrrolidone (PVP) K-25 or PVP K-12 was mixed with SYN-004 DS and dried into a film. However, upon optical evaluation of the dried films, large or small cracks were observed from 21 individual test combinations at ratios of 1:2 up to 1:5.5 (PVP:SYN-004). The reason for these cracked films was hypothesized to be an excess of protein in the mixture. In the next series of film casting experiments, SYN-004 was mixed at lower ratios with additional excipients, which included binders, plasticizers, saccharides, and combinations of these excipients, and tested in film casting experiments. In all, 123 free film castings were tested. In most of the combinations, cracks were observed in the dried film coatings, as described in Table 1. However, in a small set of mixtures of excipients and SYN-004, success was found by optical observation after drying and mechanical stress of the films. Table 1 shows that certain ratios of PVP K-12, PVP K-12 plus Pharmatose® 450 M (milled lactose), PVP K12 plus Mannitol PEARLITOL® (granulated mannitol), PVP K-12 plus sucrose, and HPC alone, resulted in the desired smooth and evenly dried films. Such smooth and even films are predictive of smooth and even layering during fluidized spray coating of an API onto sugar spheres.

Examples of the optical evaluation data for five of the film casting trials using PVP K-12:SYN-004 at several ratios are shown in Fig. 1. The images clearly show cracks at ratios of 1:1 (sample 22) and 1:0.75 (23), uneven surfaces at 1:0.5 (24), and the desired smooth and even films at lower ratios of 1:0.25 (25) and 1:0.125 (26).

Examples of the optical evaluation data for five of the film casting trials using HPC:SYN-004 at several ratios are shown in Fig. 2. The images show smooth and even film surfaces at ratios of 1:0.45 (sample 109), 1:0.40 (110), 1:0.35 (111), 1:0.30 (112), and 1:0.25 (113). Mixtures of HPC with SYN-004 at a 1:1 ratio, or higher SYN-004 amounts, resulted in film surfaces with fine cracks (Table 1), representing the upper threshold of protein concentration in order to maintain control of the drug layer quality during spray coating onto sugar spheres.

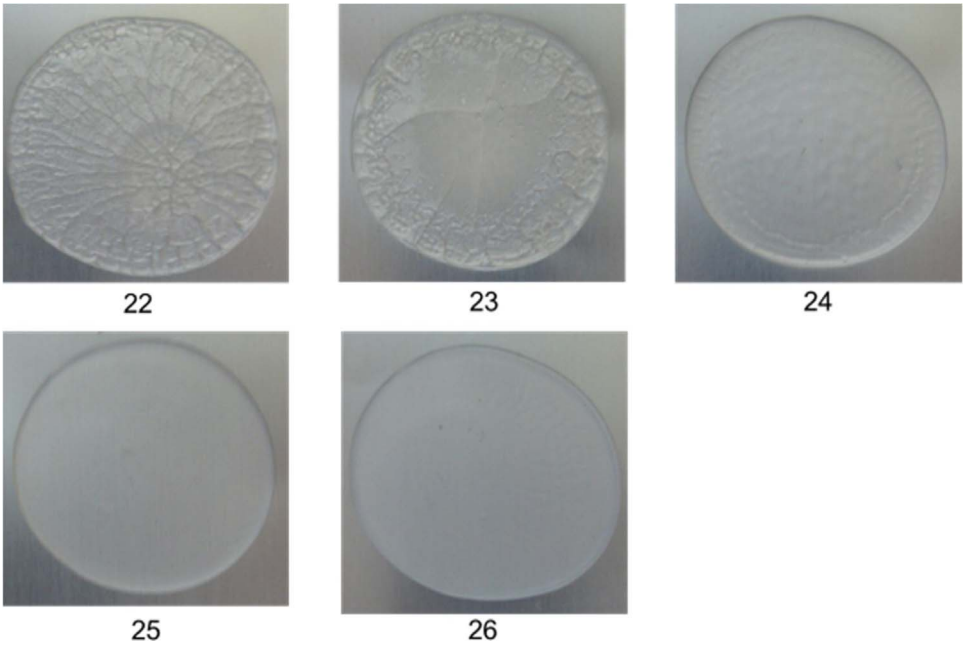

Fig. 1. Film coating images of SYN-004 and PVP K-12.
The binding excipient PVP K-12 was mixed with SYN-004 at ratios of 1:1 (sample 22), 1:0.75 (23), 1:0.5 (24), and 1:0.25 (25) and 1:0.125 (26). The films were dried to completion, stressed mechanically, and then photographed.

3.2. Development of the drug layering process

Based on the results from the film casting studies described above, several mixtures of SYN-004 with different binder excipients were selected for further testing by spraying onto sugar spheres. The solutions were prepared as described in Table 2, and sugar spheres were sprayed using the Oystar Huttlin Mycrolab Fluid Bed Dryer at an approximately 80 g batch size. Not all pellets from the spray trials were evaluable due to clogging of the spray nozzle because of stickiness or viscosity of the spray solution. The results from the spray coating trials for the drug layer are shown in Table 2.

The early results testing PVP K-25 or HPMC as binder excipients generally resulted in unsuccessful pellets due to cracked surfaces, uneven surfaces, or a clogged spray nozzle. The next series of runs included EUDRAGIT® NM 30 D, a polymer with high elasticity properties, to produce pellets with a more flexible coating. However, the addition of this polymer did not remedy the cracked surfaces observed in the absence of the polymer. Next, PVP K-12 was tried with different ratios of binder excipient to SYN-004 solution. In trial 19187/06, the resulting drug layered pellets had the desired smooth surface but still contained many cracks across the surface. Examples of SEM micrographs are shown in Fig. 3. The top two images show cracks across the surface of the pellets, and the cross-section images on the bottom show that the cracks are deep, some running down to the sucrose core.

The process yields and the analytical characterization of pellets from trial 19187/06 are shown in Table 3. These favorable process yields and preservation of enzymatic activity were promising, so additional trials founded on this base formulation were attempted. Additional excipients glycerin (19187/08) or lactose (19187/09) were added to the PVP K-12 binder, however, these spray trials were unsuccessful due to the high sticking tendencies of the pellets, and the spray runs were terminated early.

The last series of trials focused on the use of HPC as the binder excipient. The initial pellet results showed promise with smooth and even surfaces (Table 2, trial 19187/12), however the process was stopped due to sticking tendencies. Some process parameters were

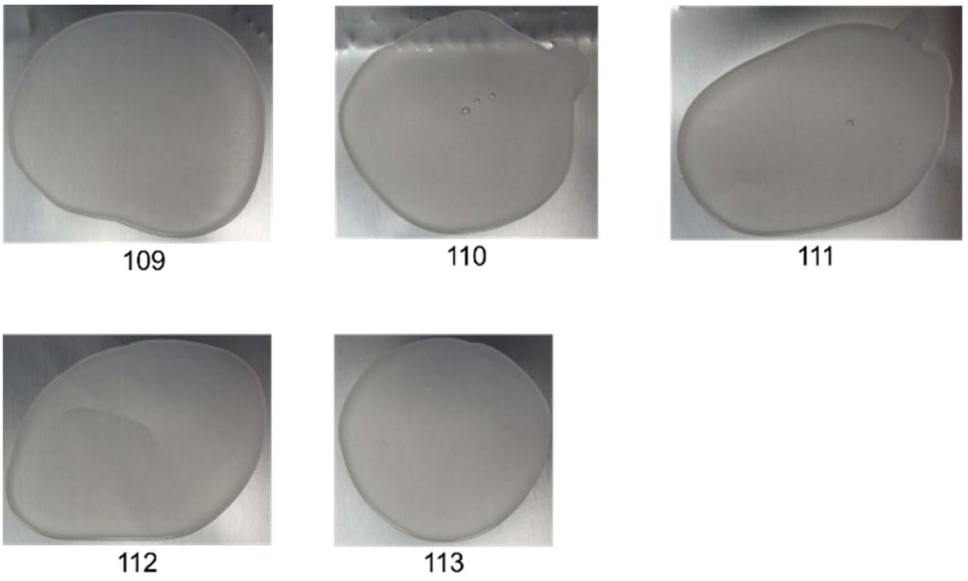

Fig. 2. Film coating images of SYN-004 and HPC.
The binding excipient HPC was mixed with SYN-004 at ratios of 1:0.45 (sample 109), 1:0.40 (110), 1:0.35 (111), 1:0.30 (112) and 1:0.25 (113). The films were dried to completion, stressed mechanically, and then photographed.

Table 2
Composition of drug layer formulations.

Composition of Drug Layer Trials						Results of spray trial (surface quality)
Trial number	Sugar spheres Amount	Excipient		SYN-004		
		Amount	Dry weight	Amount	Dry weight	
17503/70	80.0 g	4.7 g PVP K-25	4.7 g PVP K-25	256.7 g	31.6 g	Rough surface with many cracks
17930/71	80.0 g	4.7 g Sheffcoat VLV TEC	4.7 g Sheffcoat VLV TEC	256.7 g	31.6 g	Rough surface with many cracks
17930/72	80.0 g	2.3 g Sheffcoat VLV TEC; 7.8 g EUDRAGIT® NM 30 D	2.3 g Sheffcoat VLV TEC; 2.3 g EUDRAGIT® NM 30 D	233.3 g	28.8 g	Many cracks; process stopped due to high sticking tendencies
17930/73	75.0 g	2.2 g PVP K-25; 7.3 g EUDRAGIT® NM 30 D	2.2 g PVP K-25; 2.2 g EUDRAGIT® NM 30 D	220.0 g	27.1 g	Many cracks in surface visible by light microscopy
17930/74	80.0 g	20.0 g PVP K-12; 49.6 g water	20.0 g PVP K-12	90.9 g	11.2 g	Rough surface with many cracks
17930/76	80.0 g	30.0 g PVP K-12; 201.8 g water	30.0 g PVP K-12	68.2 g	8.4 g	Many cracks in surface visible by in-process stereo microscopy; process stopped
19187/06	80.0 g	11.0 g PVP K-12; 53.0 g water	11.0 g PVP K-12	200.0 g	24.7 g	Smooth surface but cracks formed
19187/07; repeated in 19187/11	80.0 g	67.4 g EUDRAGIT® NM 30 D; 3.4 g 1N NaOH; 4.0 g TEC; 2.0 g GMS; 147.0 g water	20.2 g EUDRAGIT® NM 30 D; 0.1 g 1N NaOH; 4.0 g TEC; 2.0 g GMS	23.4 g	2.9 g	Smooth, crack-free surface; spray run was repeated with similar results
19187/08	80.0 g	20.0 g PVP K-12; 4.0 g glycerin; 157.1 g water	20.0 g PVP K-12; 4.0 g glycerin	90.9 g	11.2 g	Process stopped due to high sticking tendencies
19187/09	80.0 g	8.0 g PVP K-12; 8.0 g Pharmatose 450 M; 103.3 g water	8.0 g PVP K-12; 8.0 g Pharmatose 450M	72.7 g	9.0 g	Process stopped due to high sticking tendencies
19187/12	80.0 g	22.0 g HPC; 148.0 g water	22.0 g HPC	50.0 g	6.2 g	Process stopped due to sticking tendencies, however light microscopy showed promising results; spray run repeated (19187/13) using optimized process parameters
19187/13	80.0 g	22.0 g HPC; 148.0 g water	22.0 g HPC	50.0 g	6.2 g	Slightly uneven surface, but essentially crack-free, using optimized spray process parameters
19187/15	34.0 g[a]	51.0 g HPC; 335.6 g water	51.0 g HPC	210.9 g	26.0 g	Repeat spray run of 19187/13 using optimized process parameters and conditions; the desired amount of SYN-004 was applied on spheres (45%); smooth and crack-free surfaces by SEM
19187/18	970.0 g	110.0 g HPC; 661.8 g water	110.0 g HPC	818.2 g	100.9 g	Scale-up to GPCG-3.1 equipment for 1 kg spray run; stable process but low spray rates; smooth and crack-free surfaces by SEM

[a] The drug layering process was started with 80 g of sucrose spheres. Due to the long process time, the spray process was split. After each processing day, the material was weighed and clumps were removed by sieving. The following layering steps were started with 80 g of pre-layered sugar spheres which led to a reduction in the total amount of sugar spheres in the process as the amount of spraying solution required was recalculated for each processing day.

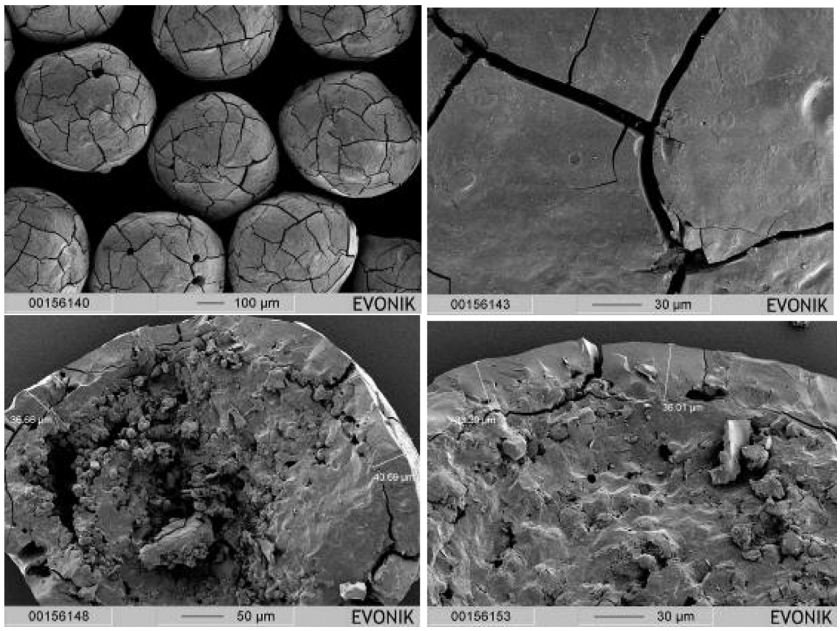

Fig. 3. Scanning electron micrographs of drug layered sugar spheres from trial 19187/06.
SYN-004 drug layered pellets from trial 19187/06 were evaluated by SEM. The top 2 images show the surface of cracked pellets; the bottom 2 images show a cross-section of the pellets after the pellets were mechanically split. The drug layer thickness was measured and annotated in the cross-section images.

Table 3
Process yields and analytical results of drug layering trials.

Process/Pellet analysis	Batch 19187/06	Batch 19187/15	Batch 19187/20
Process yield	113.9 g	106.0 g	3756.7 g
Layering yield.	98.4%	95.5%	101.4%
Theoretical assay	19.2%	21.1%	15.5%
Enzyme activity by CENTA	100.6%	97.7%	93.7%
Water content (Karl Fisher)	3.3%	2.7%	4.9%

adjusted and the formulation was repeated, which resulted in better process performance and again in pellets with smooth and even surfaces (trial 19187/13). With the success of the desired physical attributes and the higher drug loading (45% w/w), the process was replicated in trial 19187/15. The scanning electron micrographs in Fig. 4 show pellets with a smooth and even surface for the drug layer. In this prototype batch of pellets, the cross-section analysis showed 220–250 µm thickness of the drug layer, which represents 21.1% w/w of SYN-004 protein. These results demonstrated repeatability of the spray process of SYN-004 with HPC, and established the basis for the formulation and process parameters.

The process yields and the analytical characterization of pellets from trial 19187/15 are shown in Table 3. These process yields and preservation of enzymatic activity were promising, so additional trials based on this base formulation were attempted.

Finally, in trial 19187/18 the process was scaled up to approximately 1 kg using the GPCG-3.1 equipment. The spray process was stable but required a slow spray rate. The pellets in this scaled-up batch were smooth, even, and crack-free.

3.3. Development of the enteric coating process

The desired release profile for SYN-004 DP is the dissolution of enteric coated pellets in the duodenum where the bile duct empties into the small intestine. The methacrylic copolymer EUDRAGIT® L 30 D-55 was selected to provide enteric protection through the stomach, in large part because this polymer was successfully used to coat the predecessor Ipsat P1A product (Tarkkanen et al., 2009). An enteric coating trial run used the drug layered sugar sphere batch 19187/15 described above. In trial run 19187/16, 80 g of SYN-004 drug-layered pellets were used for spraying with a solution of EUDRAGIT® L 30 D-55, glycerol monostearate (GMS), triethyl citrate (TEC), polysorbate-80 (PS80), and demineralized water, as shown in Table 4. The process yield for this trial was 96.2 g with a coating yield of 81.9%. The theoretical assay of this batch yielded 14.4% w/w, the enzyme activity by CENTA assay was 94.0%, and the water content was 3.4%. When the enteric coated pellets were analyzed by SEM (Fig. 5) the smooth, even, crack-free surfaces of these pellets was evident, and the cross-sectional analysis showed the enteric coat to be 57–64 µm thick with a dense, compact character. The dissolution profile of this batch of SYN-004 pellets is presented in Fig. 6. When dissolution studies were conducted, no activity was detected after 2 h in the acid medium, indicating the enteric coat was protecting the SYN-004 enzyme. After changing to the buffer stage with pH 6.8, approximately 60% of the drug was released during the first hour and 90% was released after 4 h.

The success of the higher drug loading on sugar spheres and enteric protection displayed in batch 19187/16 satisfied the criteria to move forward with the process for the production of a larger batch required for use in nonclinical studies. An approximately 4 kg batch was produced in the GPCG-3.1 using the formulation listed in Table 5.

As demonstrated in Fig. 7, SEM analysis showed that the surface of these pellets was smooth, even, and crack-free. The cross-section analysis showed that the drug layer was 200–250 µm and the enteric coat layer was 45–50 µm. The pellets of a subsequent GMP batch produced using the same formulation and process parameters as non-GMP batch 19187/20 were sized by sieving and shown to be 89% of pellets at 1.12 mm, 8% of pellets at 1.00 mm, and 3% of pellets at 1.25 mm, demonstrating good uniformity and even sizing. The pellets were also free flowing, and product specifications were proposed based on these results. The final composition of the coated pellets is sucrose (23.3%), HPC (35.0%), SYN-004 (15.8%), buffer salts (1.6%), EUDRAGIT® L 30 D-55 (20.8%), GMS/PS80/TEC combined as PlasACRYL™ HTP 20 (3.5%).

The dissolution profile of batch 19187/20 of SYN-004 pellets is presented in Fig. 8. The results from this batch demonstrated the scalability of the small scale drug layering and enteric coating process developed, since no activity was detected after 2 h in the acid medium, and approximately 30–55% of the drug was released during the first hour and 80–95% was released after 4 h. Since this assay was performed prior to method optimization, some variability was observed

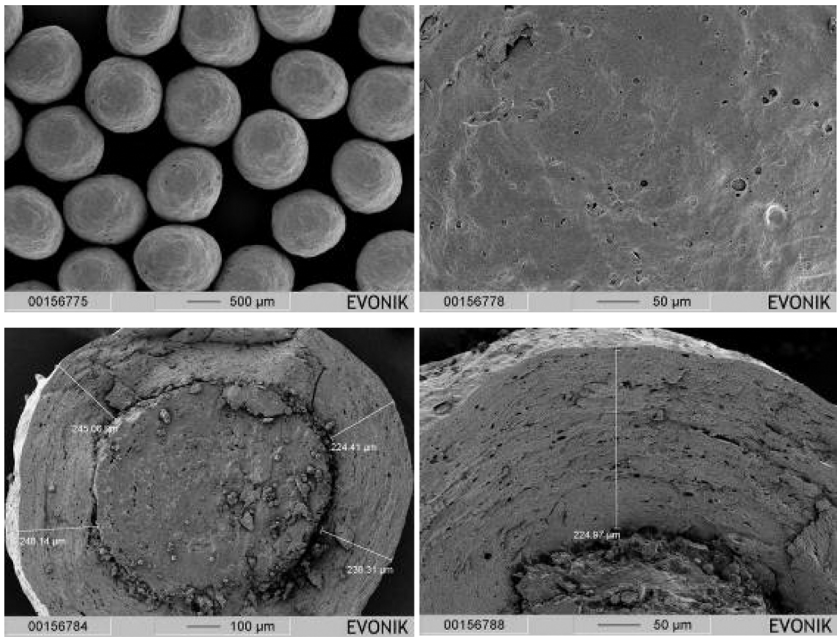

Fig. 4. Scanning electron micrographs of drug layered sugar spheres from trial 19187/15.
SYN-004 drug layered pellets from trial 19187/15 were evaluated by SEM. The top 2 images show the surface of smooth pellets; the bottom 2 images show a cross-section of the pellets after the pellets were mechanically split. The drug layer thickness was measured and annotated in the cross-section images.

among the replicate samples. Pellets from this batch were encapsulated in size 0 hard capsules and used for a nonclinical toxicology study (Kokai-Kun et al., 2016). All methods were validated prior to manufacture of GMP batches used in clinical trials.

4. Discussion

The SYN-004 delayed release DP is a capsule containing SYN-004 sugar pellets with a protective enteric coating. The DP capsule dissolves in the stomach such that the gastric protected pellets pass through the pylorus into the duodenum where SYN-004 is released when the pH rises above 5.5. The methacrylic acid copolymers have been used in numerous solid oral dosage products (Patra et al., 2017), including an investigational orally delivered insulin product (Kenechukwu and Momoh, 2016). The mechanism of action of SYN-004 β-lactamase involves the enzymatic inactivation of several susceptible β-lactam antibiotics, such as ceftriaxone and piperacillin (Kaleko et al., 2016). The clinical objective in developing this biologic drug is to protect the gut microbiome from antibiotic associated dysbiosis. Here we described the development of the SYN-004 DP formulation, using the early formulation development experience of a predecessor oral enzyme (P1A) as a starting point. The desired improvements on the previous P1A formulation were achieved since the amount of drug loaded onto sugar spheres was 16% (up from 8% in P1A) and the amount of EUDRAGIT® L 30 D-55 was reduced to 21% (down from 31% in P1A) by replacing it with HPC as the drug binder.

Our approach to the rapid development of a new and improved coated sugar pellet formulation focused primarily on the casted film study to test multiple potential excipients, rather than to provide a detailed chemical and physical analysis of protein and excipient interactions. The casted film droplet experiments were designed as a high throughput predictive tool in order to evaluate physical compatibility to evaluate alternative binder excipients and to achieve the highest feasible drug loading. A similar approach was taken in the development of a sustained release formulation of tablets containing pellets coated with ibuprofen (Abbaspour et al., 2008). By this approach, the development time was significantly reduced compared to the time and material required if the approach was to test new formulations by drug layering onto sugar pellets. Indeed, the droplet test could be performed in minutes and dried in a few hours in comparison to spray coating trials that can take many hours or days, and consume substantially more DS. In total, more than 120 droplet tests were conducted. The results of the droplet screening provided a matrix of different formulations, with qualitative ratings that supported decision making.

The first step of the drug layering formulation development was intended to identify appropriate binder excipients, and binder to SYN-004 ratios. Based on the droplet test results, it appeared that the SYN-004 solution disturbs the film formation process of the binders, leading to crack formation, even at low concentrations. The buffer salts in the SYN-004 solution could also contribute to crack formation. The most suitable binder tested, based on film forming properties and drug loading, was found to be hydroxypropyl cellulose (HPC, Klucel® EF). The best HPC to SYN-004 ratio determined from these experiments was 1:0.45 to result in a smooth, crack-free film (Fig. 2). When this mixture was used to spray the drug layer onto sugar spheres, the spray application of approximately 220% w/w was required to successfully achieve the desired SYN-004 load on the pellets. Fig. 4 shows that the pellet surface was smooth and free of cracks, which continued to be

Table 4
Composition of enteric coat formulation.

Composition of Enteric Coat Trials				Results of spray trial (surface quality)
Trial number	API Layered Sugar Spheres	Excipient		
	Amount	Amount	Dry weight	
19187/16	80.0 g	106.7 g EUDRAGIT® L 30 D-55; 1.6 g GMS; 3.2 g TEC; 1.9 g PS80; 73.8 g water	32.0 g EUDRAGIT® L 30 D-55; 1.6 g GMS; 3.2 g TEC; 0.6 g PS80	Smooth, even, crack-free surface; dense, compact layer and enteric coat in cross-section

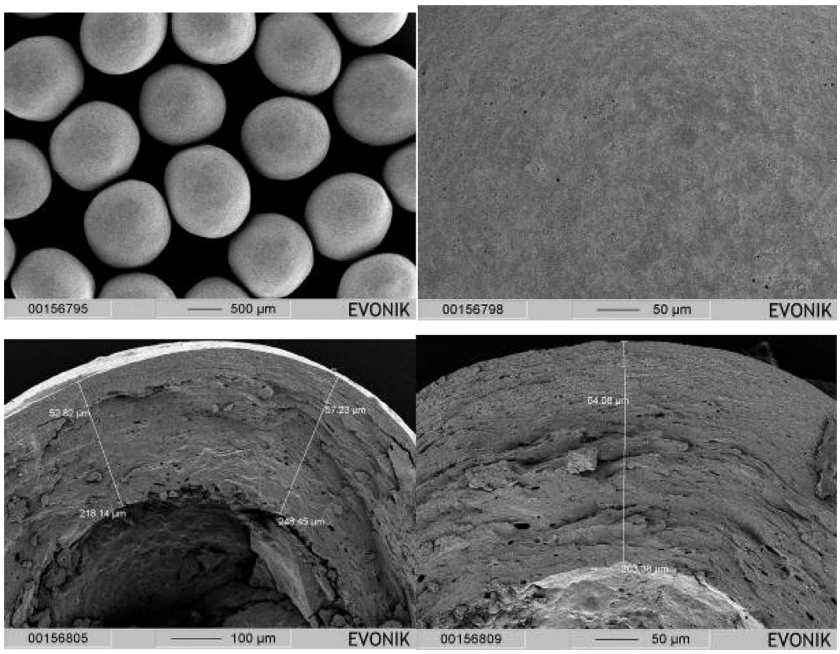

Fig. 5. Scanning electron micrographs of enteric coated sugar spheres from trial 19187/16.
SYN-004 enteric coated pellets from trial 19187/16 were evaluated by SEM. The top 2 images show the surface of smooth pellets; the bottom 2 images show a cross-section of the pellets after the pellets were mechanically split. The drug layer and enteric coat thickness was measured and annotated in the cross-section images.

observed following the enteric coat spray process (Figs. 5 and 7), indicating that this goal in SYN-004 pellet morphology was achieved.

Since EUDRAGIT® L 30 D-55 is used in the enteric coat of the SYN-004 DP, the replacement of this as a binding excipient would assure that exposure would be well below accepted IIG levels of this polymer in patients receiving SYN-004. HPC was found to be a suitable binder replacement for EUDRAGIT® L 30 D-55. It is hypothesized that the chemical interactions between the amino and carboxyl side chains on the surface of the SYN-004 enzyme and the hydroxypropyl moieties of the HPC binder facilitate the smooth surface that results as water is removed from the liquid spray during the coating process. The process also preserved the enzymatic activity throughout the coated pellet manufacture. The finding that HPC was compatible with SYN-004 as a binding excipient is an important contribution to the field of formulations for oral delivery of proteins, since it is not readily apparent which common excipients would be suitable. In fact, one report showed both stimulatory and inhibitory effects of ten polymer and 13 surfactant excipients on the activity of seven different cytochrome P450 enzymes (Martin et al., 2013), suggesting that a trial and error approach to establishing protein:excipient compatibility can be a useful screening tool in certain cases.

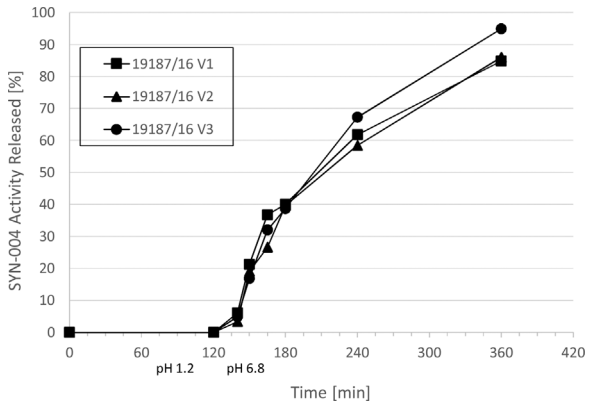

Fig. 6. Dissolution profile of enteric coated sugar spheres from trial 19187/16.
Sugar spheres from batch 19187/16 were placed in a basket for 2 h in 0.1N HCl pH 1.2, followed by 4 h in phosphate buffer pH 6.8. Samples were collected at 2 h in acid, then 15, 30, 45, 60, 120, and 240 min at pH 6.8. The samples were tested for enzyme activity using the CENTA assay and plotted on the total timeline for the dissolution analysis. Triplicate samples from the sugar sphere batch were tested individually (V1–V3).

For enteric coating of the SYN-004 layered pellets, a formulation containing EUDRAGIT® L 30 D-55 as well as the additional excipients TEC, PS80, and GMS, yielded a pellet with a smooth and crack-free surface (Table 5). This was a standard excipient mixture based on the manufacturer's guideline for the use of EUDRAGIT® L 30 D-55. The polymer application was based on weight gain to achieve a coating thickness of 40–60 µm to provide functional gastric protection. Therefore, the particle diameter was an important parameter for the enteric polymer weight gain required and SYN-004 concentration in the coated pellet. For this formulation, approximately 30% of dry polymer substance was applied on the SYN-004 drug layered pellets, which resulted in a coat thickness of approximately 40–50 µm. As shown in the dissolution profiles, the pellet formulation was well protected during the 2 h acid stage, and up to 50% of the SYN-004 activity was released within 1 h after changing to pH 6.8 buffer, and more than 80% was released within 4 h. This release profile may be predicted to support enzymatic activity for the drug in the intestine of more than 3 h. Along with the SYN-004 half-life in human chyme of at least 6 h (Kokai-Kun et al., 2017), it can be estimated that after an oral dose of SYN-004, the enzyme should be released in the duodenum and ileum, and continue to be active through transit of the small intestine.

It is important to monitor product quality attributes in the development of the SYN-004 DP, and we continue to measure particle size, dissolution, purity, identity, water content, and potency (enzyme activity) using the validated methods. Although the clinical development stage is early, there are future plans for process characterization to ensure that the process unit operations are robust and will consistently run within acceptable ranges.

It was demonstrated to be feasible to formulate SYN-004 sprayed onto sugar spheres to increase the drug loading from 8 milligrams per capsule, achieved for the Ipsat P1A β-lactamase (Tarkkanen et al., 2009), to75 mg of SYN-004 per capsule. The SYN-004 drug loading in the final enteric-coated pellets was approximately 16% w/w. This improvement in drug loading yields a higher concentration of SYN-004 in a unit dosage form which will maximize the potential of SYN-004 to degrade residual β-lactam antibiotics in the intestine, even those antibiotics such as cefotaxime which SYN-004 may have lesser enzymatic activity towards (Kaleko et al., 2016). In conclusion, the SYN-004 DP formulation described here demonstrated several significant improvements compared to

Table 5
Formulation of nonclinical batch of SYN-004 drug product.

Formulation						Results of spray trial (surface quality)
Trial number	Sugar spheres	Excipient		SYN-004		
	Amount	Amount	Dry weight	Amount	Dry weight	
19187/19	970 g	1455 g HPC; 9470.7 g water	1455 g HPC	5952.3 g	716.1 g	Smooth, even, crack-free surface; compact, dense film by cross-section SEM analysis
19187/20	2802 g (19187/19)	2568.5 g EUDRAGIT® L 30 D-55; 38.5 g GMS; 77.1 g TEC; 46.7 g PS80; 1776.9 g water	770.6 g EUDRAGIT® L 30 D-55; 38.5 g GMS; 77.1 g TEC; 15.4 g PS80	–	–	Smooth, even, crack-free surface; compact, dense film by cross–section SEM analysis

Trial 19187/19 is the drug layering run; trial 19187/20 is the enteric coating run that used the drug layered sugar spheres from run 19187/19.

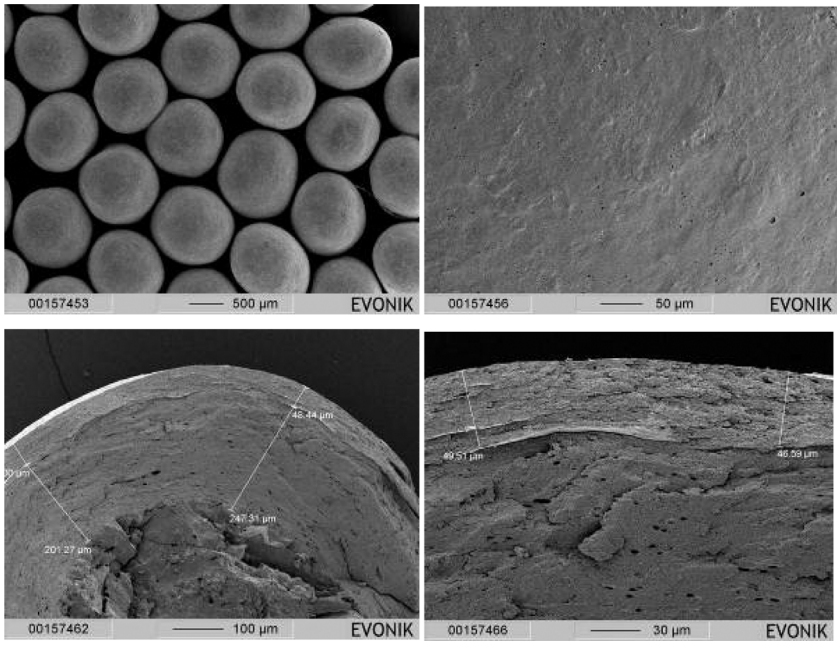

Fig. 7. Scanning electron micrographs of SYN-004 sugar spheres from trial 19187/20.
SYN-004 enteric coated pellets from trial 19187/20 were evaluated by SEM. The top 2 images show the surface of smooth pellets; the bottom 2 images show a cross-section of the pellets after the pellets were mechanically split. The drug layer and enteric coat thickness was measured and annotated in the cross-section images.

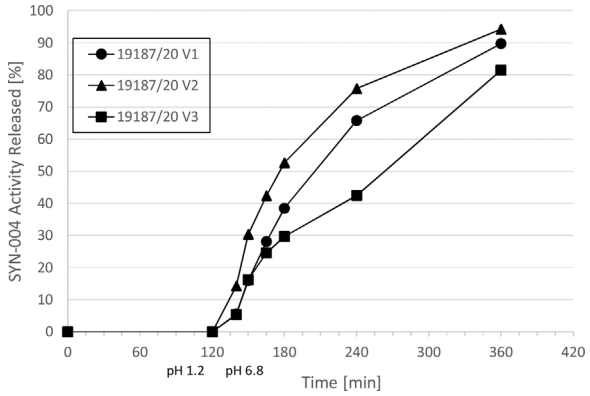

Fig. 8. Dissolution profile of enteric coated sugar spheres from trial 19187/20.
Sugar spheres from batch 19187/20 were placed in a basket for 2 h in 0.1N HCl pH 1.2, followed by 4 h in phosphate buffer pH 6.8. Samples were collected at 2 h in acid, then 15, 30, 45, 60, 120, and 240 min at pH 6.8. The samples were tested for enzyme activity using the CENTA assay and plotted on the total timeline for the dissolution analysis. Triplicate samples from the pellet batch were tested individually (V1–V3).

a predecessor oral enzyme product (P1A). These improvements provide a unique drug product designed to deliver higher doses of the active SYN-004 enzyme to release in the desired location of the small intestine where it can inactivate β-lactam antibiotics before they reach the large intestine, thereby protecting the intestinal microbiome.

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