processes

MDPI

Article

The Development of Oral Solid Dosage Forms Using the Direct-Compression Tableting of Spray-Dried Bacteriophages Suitable for Targeted Delivery and Controlled Release

Zahra Rezaie Yazdi, Mark C. Leaper * and Danish J. Malik

Department of Chemical Engineering, Loughborough University, Loughborough LE11 3TU, UK; d.j.malik@lboro.ac.uk (D.J.M.)
* Correspondence: m.c.leaper@lboro.ac.uk

Abstract: This study addresses the challenge of developing a cheap, patient-friendly alternative to antibiotics using bacteriophages for gastrointestinal applications. It explores the feasibility of manufacturing an enteric solid dosage form containing a *salmonella*-specific *Myoviridae* phage, Felix O1, encapsulated in spray-dried trehalose/Eudragit microparticles. The spray-dried powder was further formulated by combining the spray-dried microparticles with magnesium stearate to facilitate the fabrication of tablets using direct compression. The paper presents a comprehensive evaluation of the tablets with measurements of phage viability during tablet fabrication using a range of compression settings and, after tablet disintegration, dissolution and friability. Phage viability measurements were performed using storage stability testing of spray-dried powders and tablets in sealed vials at 4 °C, 20 °C and 30 °C and under different humidity conditions of 0%, 50% and 65% RH. The recommended compression force range was found to be 10–15 kN for a standard 10 mm diameter tablet. The storage of tablets at 4 °C/0% RH was found to be the most favourable condition resulting in a ~1 log loss in titre over a six-month storage period. Storage at higher temperatures and samples exposed to high levels of humidity resulted in a significant loss in phage viability. The paper highlights challenges in developing phage formulations suitable for direct-compression tableting, which afford the phages protection when exposed to temperatures and humidity levels that do not require a cold supply chain.

Keywords: antibiotic resistance; bacteriophages; direct compression; gastrointestinal infections; spray-drying; solid oral dosage forms; tablets

Citation: Yazdi, Z.R.; Leaper, M.C.; Malik, D.J. The Development of Oral Solid Dosage Forms Using the Direct-Compression Tableting of Spray-Dried Bacteriophages Suitable for Targeted Delivery and Controlled Release. *Processes* **2023**, *11*, 3146. https://doi.org/10.3390/pr11113146

Academic Editor: Roberto Pisano

Received: 20 September 2023
Revised: 23 October 2023
Accepted: 27 October 2023
Published: 3 November 2023

1. Introduction

The increasing proliferation of multi-drug resistance in Gram-negative pathogenic bacteria poses a significant global challenge both clinically and within the agricultural supply chain. It also presents problems in disaster-stricken and war-torn areas, where vaccination programmes and conventional logistics, such as a cold supply chain are not feasible. Effective low-cost prevention strategies, such as those utilising the specificity of bacteriophages for prophylactic treatment, may hold considerable promise in such situations [1–5].

Promising recent studies on using bacteriophages to combat *Pseudomonas aeruginosa* have used dry powder inhalers, topical applications and enteric-coated tablets as drug delivery routes [6–9], improving on the vial-based methods of storage and administration previously used. The jump from the very limited vial-based systems to dry powder systems has been facilitated by encapsulating the phages within a sugar matrix using spray-drying; the amorphous sugar performs many of the tasks normally provided via water and enables fully stable phages to exist as part of a dry powder. A logical development from these studies is to examine the efficacy of using phages delivered using conventional tablets produced using direct compression, which poses a number of challenges. The

phages need to be stable in the formulation in the absence of a cold supply chain [10]. Bacteriophages may also encounter chemical and physical stresses during processing which may be detrimental to phage activity, resulting in loss of potency. The formulation of phages needs to ensure the reliable delivery of high doses of phages at the site of infection and afford protection to phages, which are susceptible to stomach acid and proteolytic damage during transit through the gastrointestinal tract [11]. The process of manufacturing oral dosage forms needs to be scalable and relatively low-cost [12].

The spray-drying of bacteriophages is increasingly recognised as a low-cost and scalable process technology for manufacturing phage drug products in a suitable dry powder form. It has previously been shown that the spray-drying of bacteriophages using formulations containing the sugar trehalose and a pH-responsive synthetic polymer may allow high encapsulation yields, affording phage acid stability and allowing pH-triggered release in a controlled manner in response to changes in environmental pH, such as in the digestive tract [13,14]. It has also previously been demonstrated that encapsulated phages survive the process of direct compression and can be processed into tablets which are superior in terms of acid protection compared with powders [13]. Tablets are the preferred form of oral delivery, as they have a high level of patient acceptability and compliance. They provide an easy means of accurate dosage and are simple to administer. Direct compression is a simple method for making tablets; however, several factors need careful consideration for the successful manufacture of phage-encapsulated tablets which heretofore have not been systematically evaluated in the literature. The aim of the present study was to spray-dry a *Salmonella*-specific phage (the *Myoviridae phage Felix O1*) using two different pH-responsive polymers, Eudragit L100® and S100®, thereby allowing the potential targeting and controlled release of encapsulated phages either in the upper or the lower intestinal tract, respectively. The effect of different compression forces used to fabricate the tablets on phage viability, tablet disintegration and friability were evaluated. The resistance to acid exposure and storage stability of the encapsulated phages in the powders and tablets were evaluated. The role of residual moisture in the spray-dried powders and its effect on phage viability during storage were investigated in order to improve phage drug product stability in the absence of a cold supply chain.

2. Materials and Methods

2.1. Preparation of the Active Ingredient, Bacteriophage Felix O1

The preparation of the phage lysate followed the method used in previous studies [13], using *Salmonella enterica ATCC19585* and *phage Felix O1*; these were obtained from LGC standards (Teddington, Middlesex, UK) and the University of Nottingham, respectively. The Salmonella were grown in 50 mL cultures (BHI, Oxoid, Basingstoke, UK) inoculated with 500 µL of an overnight starter culture (37 °C). A log-growth phase culture at an optical density (OD_{550}) of 0.2 was measured using a spectrophotometer (UV Mini 1240, Shimadzu, Kyoto, Japan) and typically equated to a viable cell density of 10^8 colony-forming units (CFU mL^{-1}); this system was then infected with 50 µL of the phage *Felix O1* at a multiplicity of infection (MOI) of 0.01 and grown overnight in a shaking incubator (Certomat® BS-1) at 37 °C and 150 RPM. The phages were subsequently harvested via the removal of un-lysed cells and cell debris using centrifugation at 2000× *g* for 15 min. The supernatant was filtered using a 0.2 µm sterile in-line syringe filter (Millipore, Watford, UK), and the final phage lysate was stored under refrigerated conditions at 4 °C.

2.2. Phage Titration Using Double-Layer Plaque Assay

The phage titration was evaluated using a double-layer plaque assay, as in previous works [13,15]. In a sterile tube, typically, 5 mL of BHI top agar (BHI broth, Oxoid, Basingstoke, UK with 0.5% Bacteriological Agar No. 1, Oxoid, Basingstoke, UK) was mixed with 5 mL of a salt solution (400 mM of $MgCl_2$ and 100 mM of $CaCl_2$). Then, 10 µL of *salmonella* culture was added to this mixture, poured over a BHI agar plate and dried under a laminar flow hood. Next, 10 µL phage samples were serially diluted 10-fold over

the range 10^{-1} to 10^{-8} and spotted in triplicate on a bacterial lawn overlay containing *salmonella*. The plaque-forming units (PFU) were counted after overnight incubation for 24 h at 37 °C. Data were represented as PFU per gramme of spray-dried solid.

2.3. Spray-Drying of Bacteriophage Felix O1 in pH-Responsive Formulations

Two pH-sensitive polymers were used in the formulation, Eudragit S100 (CAS25086-15-1) and L100 (CAS 25086-15-1); these were kindly donated by the manufacturer Evonik Germany. These polymers are versions of polymethacrylic acid co-methyl methyacrylate, which will dissolve in solutions with a pH greater than 6 and will protect the phages from the acidic conditions found in the stomach. The formulation also contained D-Trehalose dihydrate purchased from Fisher Scientific (Loughborough, UK). Two different formulations were used for spray-drying—these were PS21-S100 containing 2% (*w*/*v*) S100 and 1% (*w*/*v*) trehalose and PS21-L100 containing 2% (*w*/*v*) L100 and 1% (*w*/*v*) trehalose; 4 g of each polymer was dissolved fully in a solution of 4 mL of 4M NaOH and 36 mL of de-ionised water. The final solution pH was between 6.5 and 7. This polymer solution was diluted using de-ionised distilled water to a final polymer concentration of 2% (*w*/*v*), and 1% (*w*/*v*) trehalose was added to the solution. Then, 1 mL per 100 mL of solution of the high-titre phage Felix O1 (~10^{10} PFU/mL) was added to the polymer–trehalose solution prior to spray-drying. The ratio of trehalose to Eudragit had been determined in previous optimisation studies which examined the impact of spray-drying on the phages [13]. Trehalose protects the phages from heat and moisture migration during the spray-drying process and subsequent storage.

The spray-dried powders were produced using a LabPlant^{TM} SD-06 Spray Dryer (LabPlant, Hunmanby, UK) fitted with a 0.5 mm two-fluid nozzle. The inlet temperature of the equipment was set to 150 °C, achieving an outlet temperature of 80 ± 2 °C. The flow rate of the solution was set to 280 mL h^{-1} with an air speed of 4.3 ms^{-1}. Prior to spray-drying, water was sprayed until the desired outlet temperature was achieved, after which the phage-containing polymer solution was spray-dried. The spray-dried product containing the phages was collected in a dry collection bottle located below the cyclone. The viable phage concentration in the powder (PFU/g) was determined by dissolving ~0.3 g of powder in simulated intestinal fluid (SIF, Sorenson's buffer, pH 8), and the phage was serially diluted and plated using a double-overlay assay described previously.

2.4. Assessing the Water Content and Morphology of Spray-Dried Powder Using Thermogravimetric Analysis (TGA)

TGA experiments were performed on a TGA/DSC 1 from Mettler Toledo instruments at a heating rate of 1 °C/min from 30 to 125 °C using 70 µL alumina crucibles.

2.5. Tablet Production Using Direct Compression

A Riva Minipress MII (UK) single-station tablet press machine was used to produce tablets in a direct-compression process using just the spray-dried powder and a 2% (*w*/*w*) magnesium stearate lubricant (Sigma Aldrich, Gillingham, Dorset, UK). The tablets were circular with a conventional breakline of a diameter of 1 cm, a thickness of 0.5 cm and an average weight of 0.29 g. The tablets were produced using distinct controlled compression forces of 5, 15, 20, 25, 30 and 35 kN for each formulation.

2.6. Dissolution Testing of Formulated Powders and Tablets

When administered, these tablets would first pass into the stomach at a low pH for 2 h and then exit into the small intestine, the intended site of action, at a pH of 8. This meant that the dissolution test method needed to take this into account. The tests were also performed for both tablets and powders. For tablets, 900 mL of a dissolution medium (±1%) was poured in the Caleva ST7 dissolution tester apparatus (USP apparatus 2), and the dissolution medium was equilibrated to 37 ± 0.5 °C. One dosage unit was placed at the bottom of the apparatus, ensuring the exclusion of air bubbles from the surface of the tablets. The apparatus was covered, and samples were taken every 30 min from each container, and

followed the protocol;as shown in Figure 1. Equal volumes of fresh dissolution medium at 37 °C were replaced after each sampling. Phage titration was checked for every time point according to the *British Pharmacopeia* standards. To perform the equivalent test for powders, 0.29 g of powder was placed inside a centrifuge tube and dissolved in a prewarmed pH 8 simulated intestinal fluid (SIF) Sorensen's buffer. The samples were placed in the incubator at 37 °C for 3 h, and 10 µL was taken every 30 min and diluted using the method explained earlier. The results were analysed using a double-layer plaque assay.

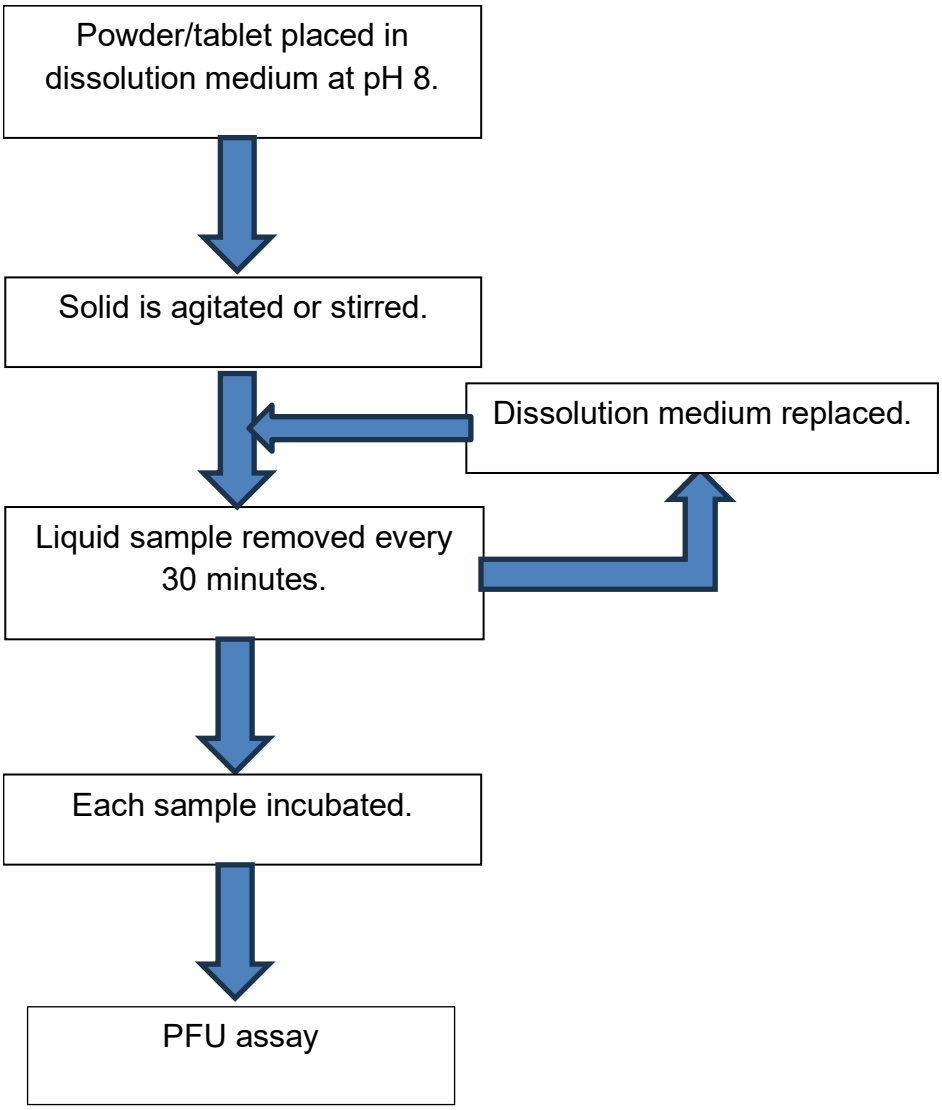


Figure 1. Schematic representation of the dissolution testing. Tablets were placed in the bottom of the vessel containing the medium (pH 8), and the apparatus was operated. Similarly, powders were placed inside a tube and inside a shaking incubator. For both tablets and powders, the medium was maintained at 37 ± 0.5 °C, and samples were taken every 30 min.

To assess the gastro resistance of the encapsulated phage for each formulation, the phage titration of powders and tablets was measured after exposure to the acidic medium (pH 1 and 2) and once released at pH 8. The phage titration of each sample was then compared to those samples that had only been released at pH 8 (no acid exposure). The samples were exposed to the acid solution of NaCl and HCl for 2 h, followed by complete dissolution using Sorenson's buffer at pH 8.

2.7. Tablet Friability Tests

The tablet friability/abrasion testing process followed international standards [16]. Each measurement used a batch of whole tablets with a mass totalling around 6.5 g. The tablets were carefully dusted, weighed and placed inside the drum of an Erweka friability tester (model no. TA 120). The device rotated the drum at 25 ± 1 rpm 100 times. The weight loss percentage per batch was calculated, with the tests being done in triplicate. According to BP (2019), after the test, there should not be cracked, cleaved or broken tablets, and the total weight loss of the sample should not be more than 1%. This was achieved for all tablet formulations across the full range of compaction values.

2.8. Tablet Disintegration Tests

An Erweka disintegration tester (model type ZT31) was used to determine compliance with the limits of disintegration according to international standards [16]. The apparatus consisted of a basket-rack assembly, a 1000 mL, low-form beaker for the immersion fluid and a thermostatic arrangement for heating the fluid. Tablets were placed in each of the six tubes of the basket, and the apparatus was operated such that the tablets were raised and lowered whilst placed on a wire mesh that was fully immersed in simulated gastric fluid (SGF, 0.2 M NaCl, at pH 1 adjusted using 1 M HCl). The liquid medium was maintained at 37 ± 2 °C. Once the temperature of the medium had reached 37 ± 2 °C, the apparatus was operated for 2 h. The weight of the tablets was measured at the start and after the disintegration testing run of 2 h (following drying in an oven for 24 h at 50 °C). The equivalent amount of powder for each formulation was placed in 15 mL falcons, exposed to pH 2 (0.2 M NaCl) for 2 h in a shaking incubator. The powder or tablets that survived the disintegration test were subsequently dissolved in simulated intestinal fluid (SIF, pH 8) and serially diluted, and viable phage counts were obtained using the double-overlay agar method using spot assays. All measurements were repeated in triplicate.

2.9. Statistical Analysis of Results

A comparison of sample means using 2 sample *t*-tests was carried out using the statistical software IBM SPSS.

3. Results

3.1. Assessing the Effects of Processing on Phage Viability

Prior to any detailed study of the formulations, it was necessary to check that the processes involved in making the solid dosage forms did not affect the phage viability. The phage counts for PS21-S100 and PS21-L100 before spray-drying, theoretically determined from the initial mixture, and the counts measured from the powder after spray-drying and then dissolution in simulated intestinal fluid (SIF) were compared and showed that the spray-drying process did not result in significant phage viability loss.

The effect of compaction on the phages within the spray-dried powder was also checked; Figure 2 shows that up to 15 kN, the phages were unaffected, with a progressive deterioration in numbers between 15 kN and 35 kN for both formulations. Figure 3 is an SEM of a cross-section of tablets produced at 15 kN and 35 kN, and it clearly shows that the individual particles produced from spray-drying were still intact within the tablet structure.

3.2. Initial Dissolution Analysis of Powders and Tablets Containing PS21-S100 and PS21-L100 in Simulated Intestinal Fluid (SIF)

Both powders and tablets were tested on the basis of phage dissolution; initially, this was done in SIF at pH 8 for PS21-S100 and pH 6 and 7 for 3 h for PS21-L100 and achieved a complete release within the time period compared to the theoretical value calculated from the initial solution. Figure 4 shows detailed time-based phage release profiles from the powder and tablet samples exposed to SIF with phage release completing over a period of 180 min.

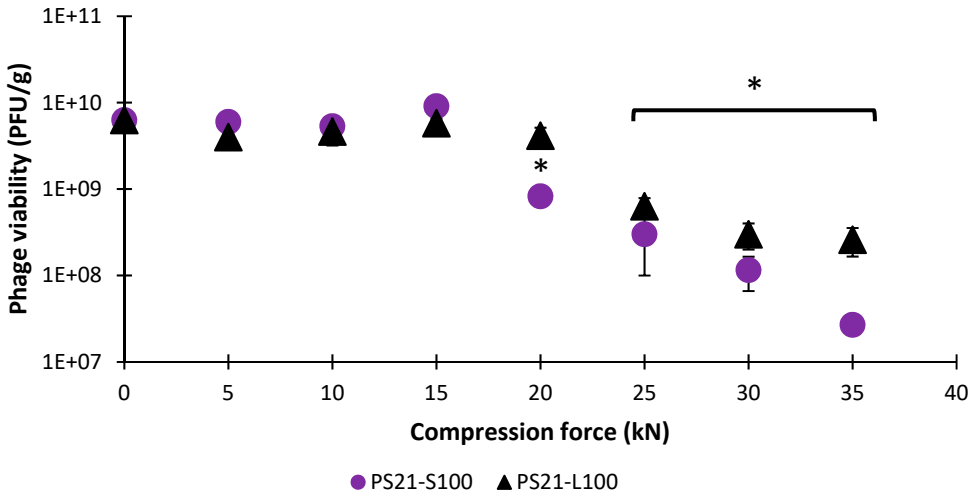


Figure 2. The influence of the applied compaction pressure on the viability of encapsulated bacteriophage. Error bars represent one standard deviation. * Indicates a statistically significant difference in sample means at a particular compaction pressure with the phage titre in the original spray-dried powder ($p < 0.05$) using a two-sample *t*-test. All measurements were performed in triplicate (n = 3).

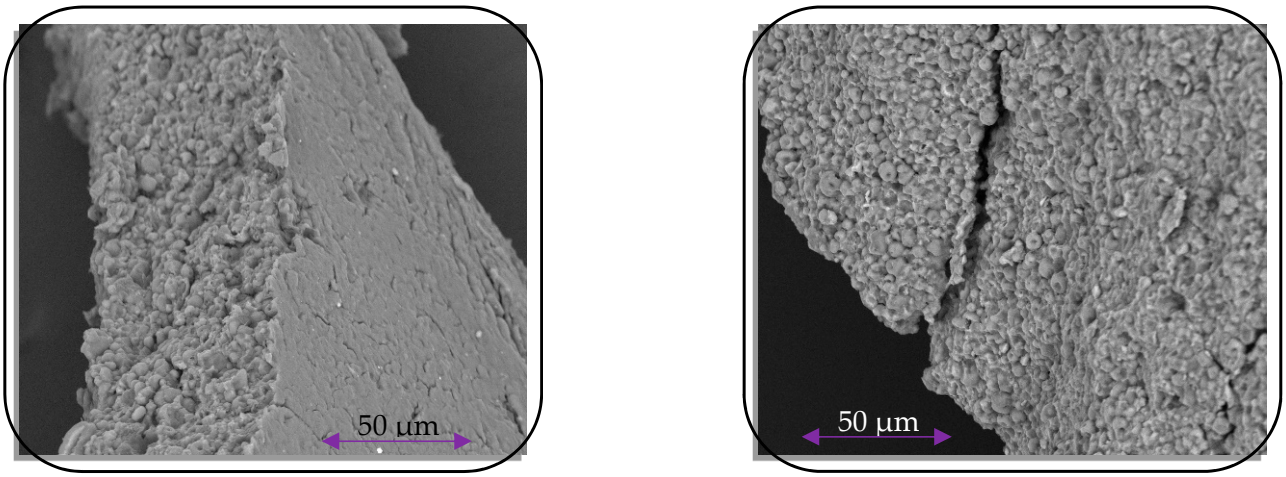


Figure 3. SEM images of compressed tablets of PS21-S100 (**i**) at 15 kN and (**ii**) 35 kN, which did not show any apparent damage to the particles.

3.3. Analysis of the Effect of Simulated Gastric Fluid (SGF) on Phage Viability

In order for the formulations to achieve the desired efficacy, it was necessary to also check that the phages were protected in an acid environment prior to release to simulate the passage through the stomach. To achieve this, both powders and tablets containing the two formulations were exposed to simulated gastric fluid (SGF) at both pH 1 and pH 2 for two hours prior to the SIF dissolution test. The results in Figure 5 show that the spray-drying alone provided some protection, with reductions of up to one order of magnitude; however, when the formulations were in tablet form, the phages emerged unscathed from the acid conditions and achieved the same dose as the control.

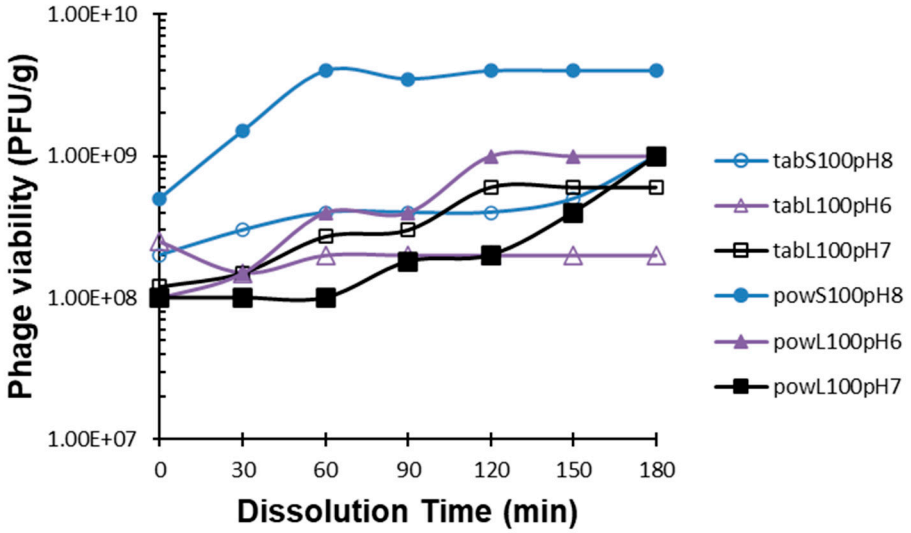


Figure 4. PS21-L100 released in SIF at pH 6 and 7 and PS21-S100 released in SIF at pH 8. Both powder (pow) and tablet (tab) forms were investigated. The tablets were produced with 2% w/w magnesium stearate and a compaction force of 5 kN. Analysis of the results indicated a significant difference in the means of powders released at pH 7 and the tablets released at pH 6 and 7 ($p < 0.05$) using a two-sample t-test. All measurements were performed in triplicate ($n = 3$).

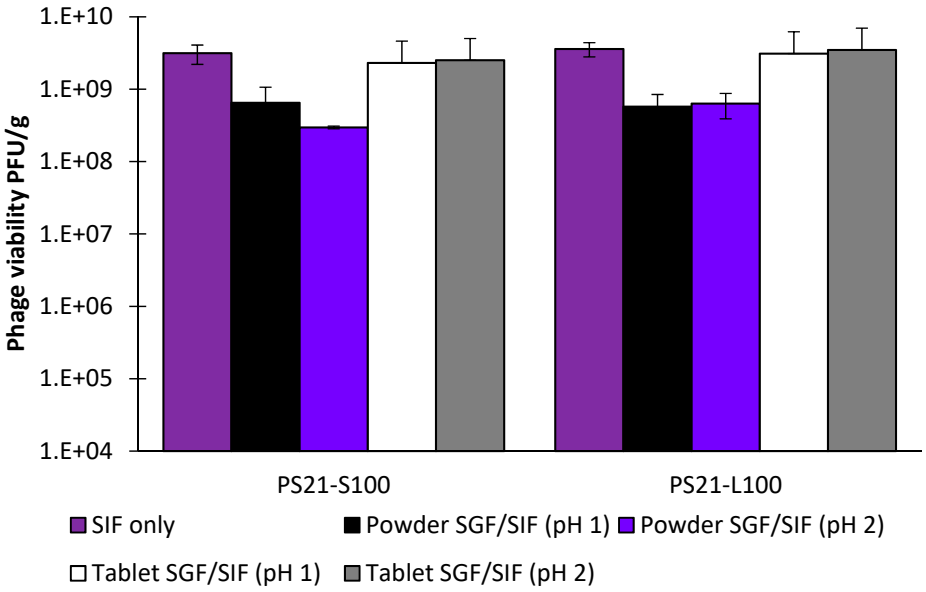


Figure 5. Exposure of PS21-S100 and PS21-L100 formulation powders and tablets to acidic conditions pH 1 and pH 2 using SGF for 2 h, followed by a complete release in SIF at pH 8 for 3 h. It indicates no significant difference in the means of samples exposed to SIF only compared to those exposed to SGF followed by SIF ($p > 0.05$) using a two-sample t-test. Error bars represent one standard deviation; all measurements were performed in triplicate (n = 3).

Having obtained dissolution data on the tablets, which indicated that encapsulating the phages within a trehalose/Eudragit spray-dried structure provided protection from acidic conditions, the disintegration characteristics under similar conditions were determined, as shown in Figure 6. The data show that compression force during tablet manufacture did not significantly affect the disintegration characteristics of the tablets, with PS21-S100 tablets disintegrating slightly less than PS21-L100. There was a considerable breakup of the tablets during this process, but the dissolution data from Figure 5 show that the PFU/g

values were unaffected, and the protection from acidic conditions was still provided by particle agglomerates; these structures were an improvement on powder alone.

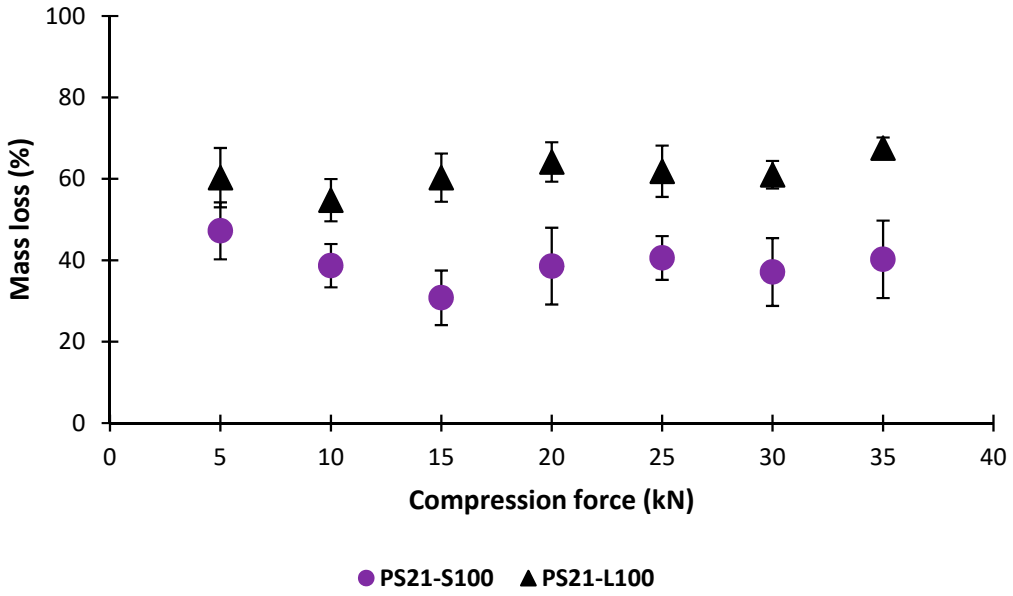


Figure 6. Tablet disintegration and the total mass loss of each tablet for PS21-S100 and PS21-L100 after 2 h of exposure to SGF (pH 1). Error bars represent one standard deviation. All measurements were performed in triplicate (n = 3). No difference was observed in the mean mass loss of the tablets produced at 5 kN compaction pressure and the tablets manufactured at higher forces.

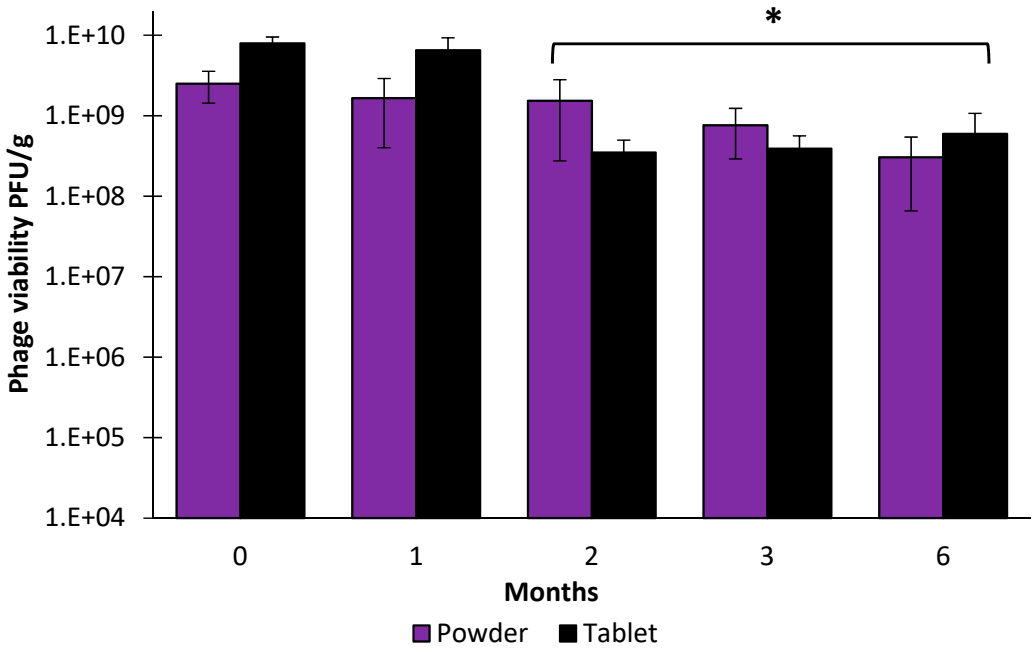
3.4. Stability Studies of Powders and Tablets Containing PS21-S100 and PS21-L100

Having established the efficacy of the two formulations immediately after production, stability tests were performed to analyse how the tablets and powders provided protection to the phages under a range of temperatures and relative humidity levels over storage times of several months. This was quantified by measuring the PFU/g of the dosage forms and the moisture content using thermogravimetric analysis (TGA) at monthly intervals to show the relationship between formulation, storage time, moisture uptake and phage viability.

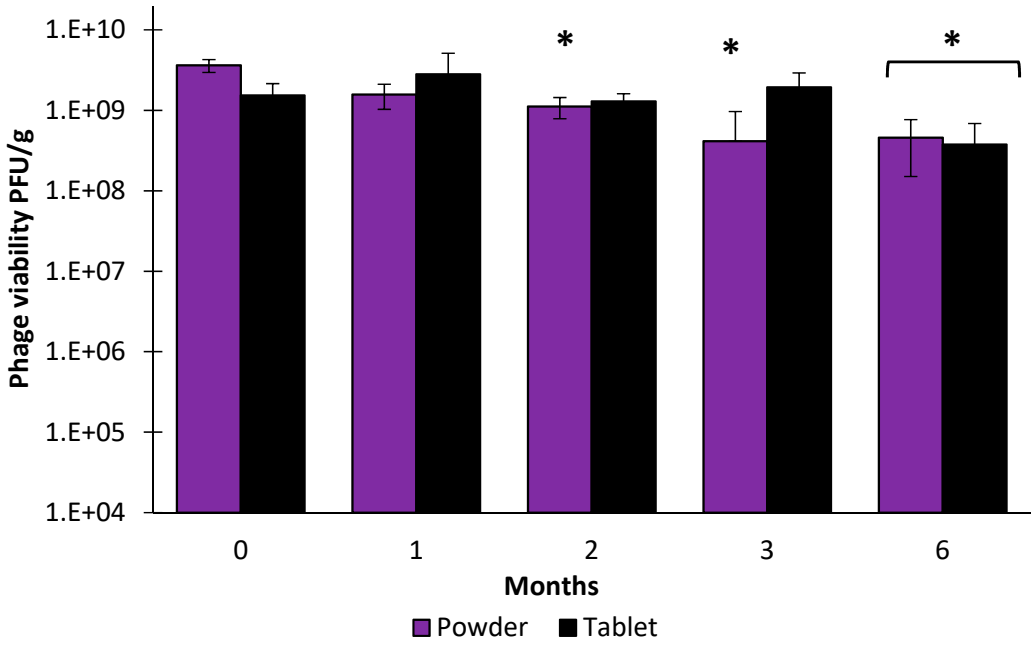
After production, the samples were allowed to cool to room temperature and relative humidity (20 °C and 50% RH); they were then placed in vials with lids sealed with wax prior to being placed in a test chamber. The conditions were 4 °C and 0% RH (fridge conditions), 20 °C and 50% RH (ambient conditions) and 30 °C and 65% RH. To investigate the effect of humidity, samples fully exposed to 30 °C at both 65% RH and 0% RH were also examined.

Figure 7 shows the ability of both formulations in tablet and powder form to protect phages over a period of 6 months when sealed and stored under fridge conditions. Figure 8 also shows how there was negligible moisture uptake for all dosage forms during this time.

Samples were also tested at 20 °C, at which PS21-L100 still protected the phages for 3 months, as shown in Figure 9; this result was reflected in the moisture content measurements shown in Figure 10, in which there was little moisture adsorption over the first 3 months in both powder and tablets, followed by a subsequent increase and decline in the value of PFU/g. By contrast, despite having a lower initial moisture content, PS21-S100 did not provide the required protection for the stored phages, with the powdered phages being more vulnerable than those in the tablets.



(**a**)



(**b**)

Figure 7. Stability studies results for (**a**) PS21-S100 and (**b**) PS21-L100 formulations in powder and tablet forms. Samples were stored for six months at 4 °C in closed and sealed vials. Error bars represent one standard deviation. * Indicates a significant difference between the mean of the tested parameter in comparison to the mean value at 0 months ($p < 0.05$) in a two-sample *t*-test All measurements were performed in triplicate (n = 3).

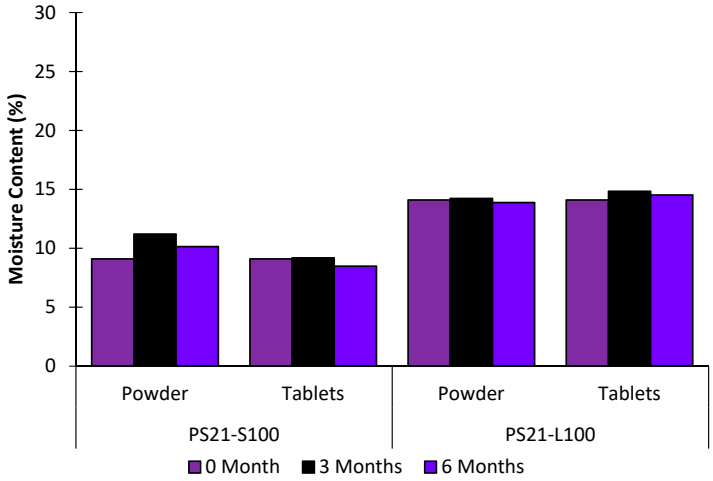


Figure 8. Moisture analysis of powders and tablets for PS21-S100 and PS21-L100 stored for 6 months at 4 °C in closed and sealed vials. The glass vials were opened for every sampling time. Once the sample was removed, the vials were then closed and resealed using parafilm.

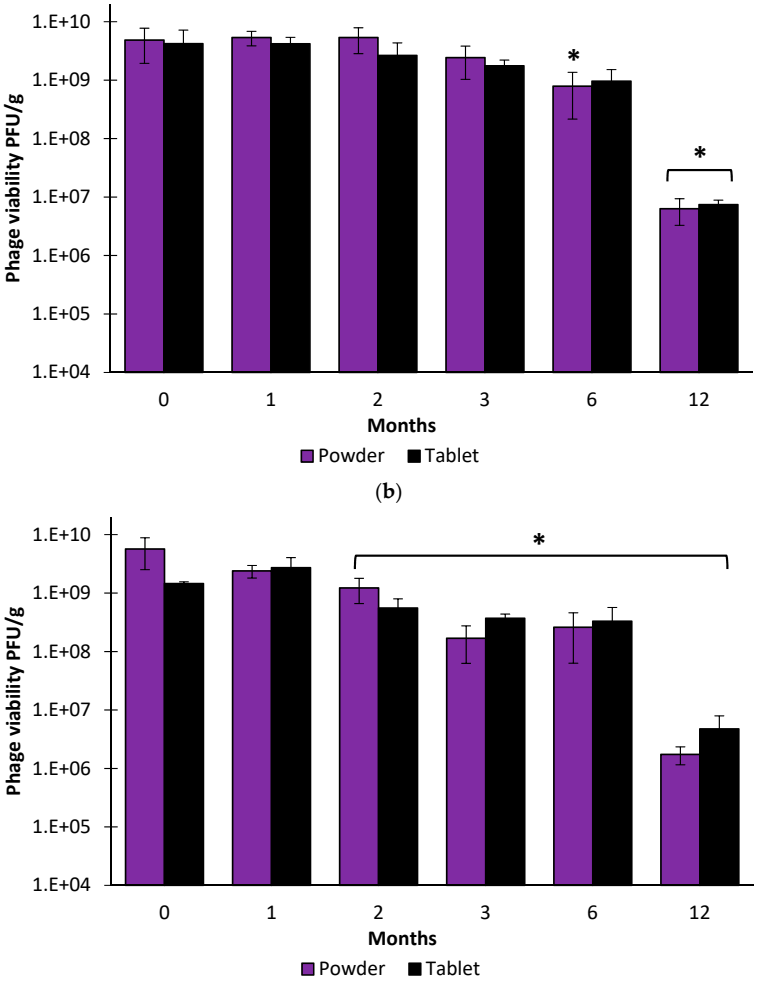


Figure 9. Stability studies carried out at 20 °C for 12 months on powders and tablets of (**a**) PS21-S100 and (**b**) PS21-L100 formulations in closed and sealed vials. Error bars represent one standard deviation. * Indicates a significant difference between the mean of the tested parameter in comparison to the mean value at 0 months ($p < 0.05$) in a two-sample *t*-test. All measurements were performed in triplicate (n = 3).

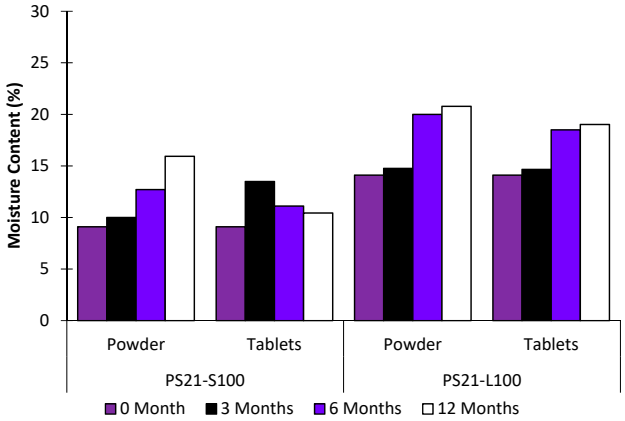
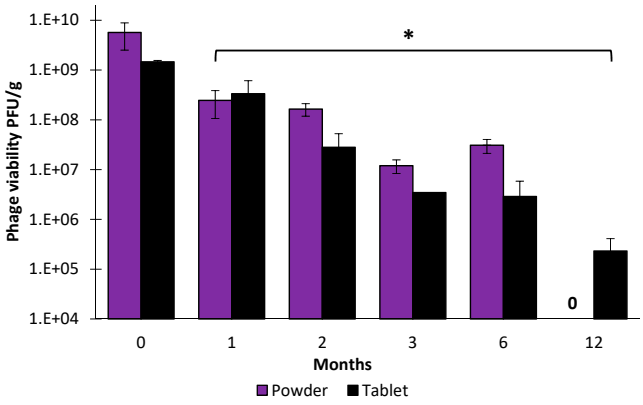
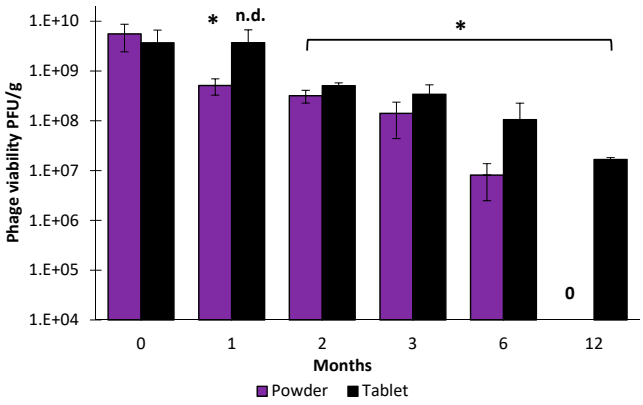


Figure 10. Moisture analysis of powders and tablets for PS21-S100 and PS21-L100 stored for 12 months at 20 °C in closed and sealed vials. Glass vials were sealed again after every sampling time.

Figures 11 and 12 show the phage degradation and moisture uptake of the two formulations in vials that were sealed and stored at 30 °C and 65% RH. Despite being stored in a sealed vial, there was very rapid degradation of the powdered phages, particularly after 3 months. Phages within the powdered formulations were not detectable after 12 months.



(**a**)



(**b**)

Figure 11. Stability studies carried out at 30 °C and 65% RH for 12 months on powders and tablets of (**a**) PS21-S100 and (**b**) PS21-L100 formulations in closed and sealed vials. Error bars represent one standard deviation. * Indicates a significant difference between the mean of the tested parameter in comparison to the mean value at 0 months ($p < 0.05$) in a two-sample *t*-test. All measurements were performed in triplicate (n = 3).

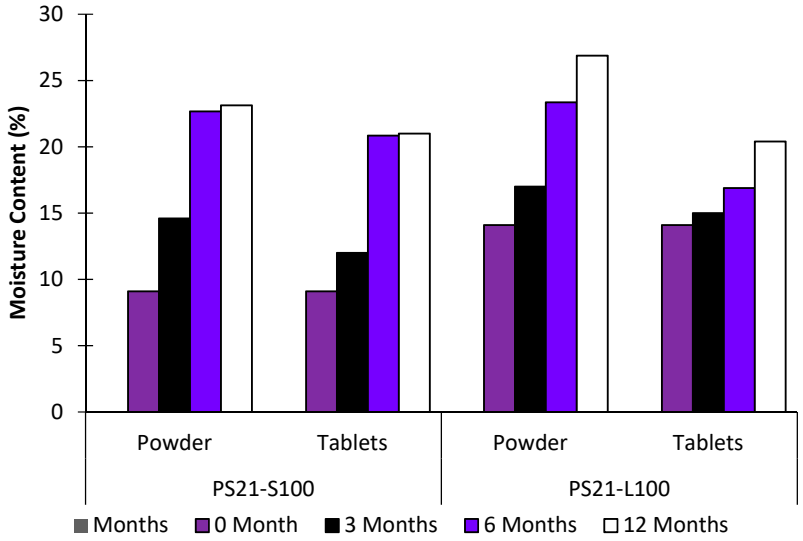


Figure 12. Moisture analysis of powders and tablets for PS21-S100 and PS21-L100 stored for 12 months at 30 °C + 65% RH in closed and sealed vials. After every sampling time intervals, the vials were immediately sealed to prevent additional moisture uptake.

In order to assess the role of humidity under these conditions, two further sets of tests were performed which compared the effects of 0% RH and 65% RH at 30 °C on exposed tablets and powders containing PS21-S100 and PS21-L100 formulations. Figures 13 and 14 show how the PFU/g value decreases within 3 months at 65% and 0% RH respectively, with Figure 15 showing the changes in moisture content. This data showed that the prolonged exposure of the phages to this temperature did cause some degradation, but the addition of high humidity greatly accelerated this process, particularly with L100 in powder form.

3.5. Tablet Friability

Despite some formulations showing promising degrees of efficacy, it was important to establish that the tablets produced were robust enough to withstand automatic handling and packaging. A friability test, as described in the previous methods section, was performed on tablets of both the PS21-S100 and PS21-L100 formulations made with compaction forces of 5 to 35 kN. Both tablet formulations at compaction of 5 kN showed up to a 20% loss by mass; however, at 10 kN and higher compaction, no loss was measured. This shows a feasible operating window to be within the range of 10–15 kN of compaction force.

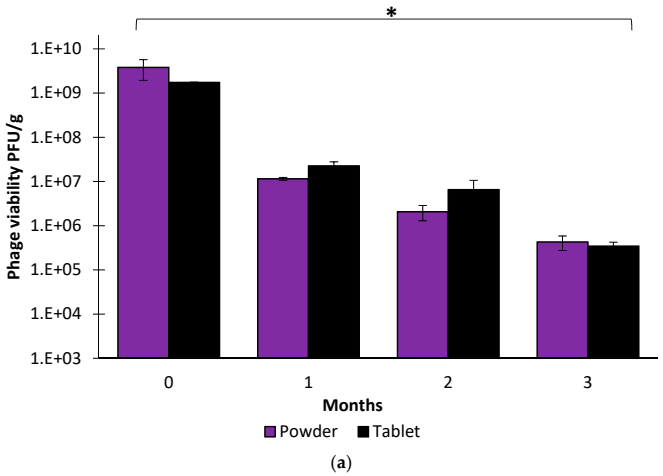
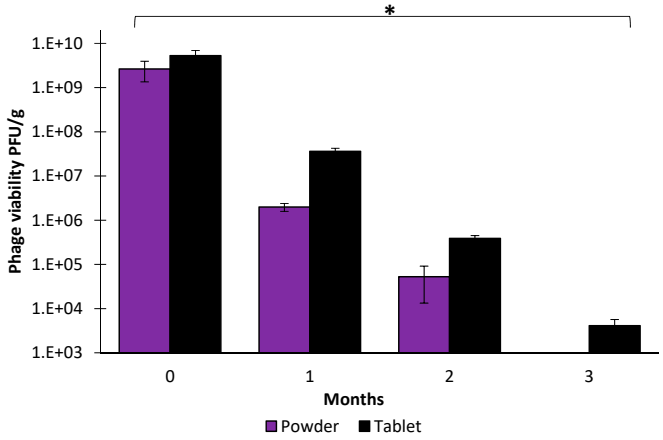
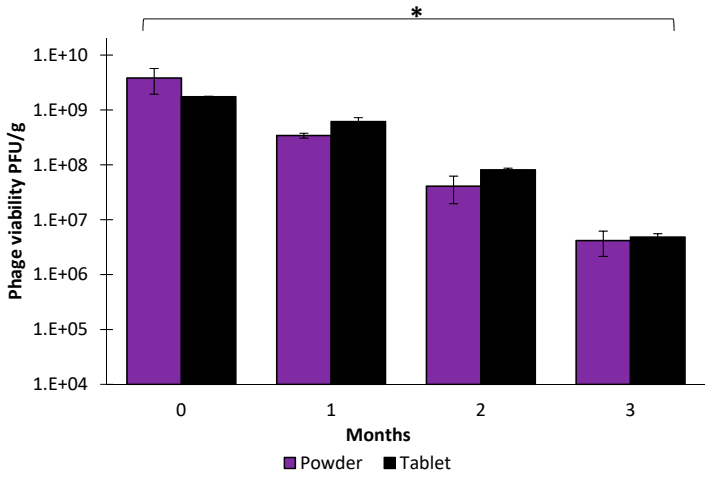


Figure 13. *Cont.*

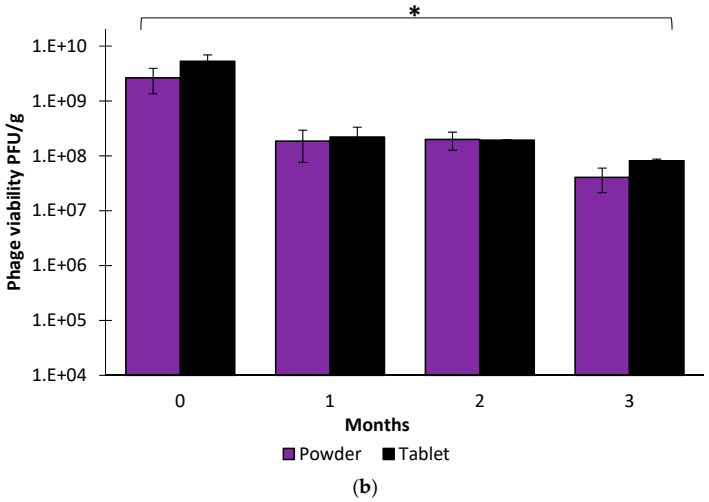


(**b**)

Figure 13. Storage of (**a**) PS21-S100 and (**b**) PS21-L100 formulations in powders and tablets for 3 months at 30°C and 65% RH with no protection. Error bars represent one standard deviation. * Indicates a significant difference between the mean of the tested parameter in comparison to the mean value at 0 months ($p < 0.05$) in a two-sample *t*-test. All measurements were performed in triplicate (n = 3).



(**a**)



(**b**)

Figure 14. Storage of (**a**) PS21-S100 and (**b**) PS21-L100 in powders and tablets for 3 months at 30 °C and 0% RH. Error bars represent one standard deviation. * Indicates a significant difference between the mean of the tested parameter in comparison to the mean value at 0 months ($p < 0.05$) in a two-sample *t*-test. All measurements were performed in triplicate (n = 3).

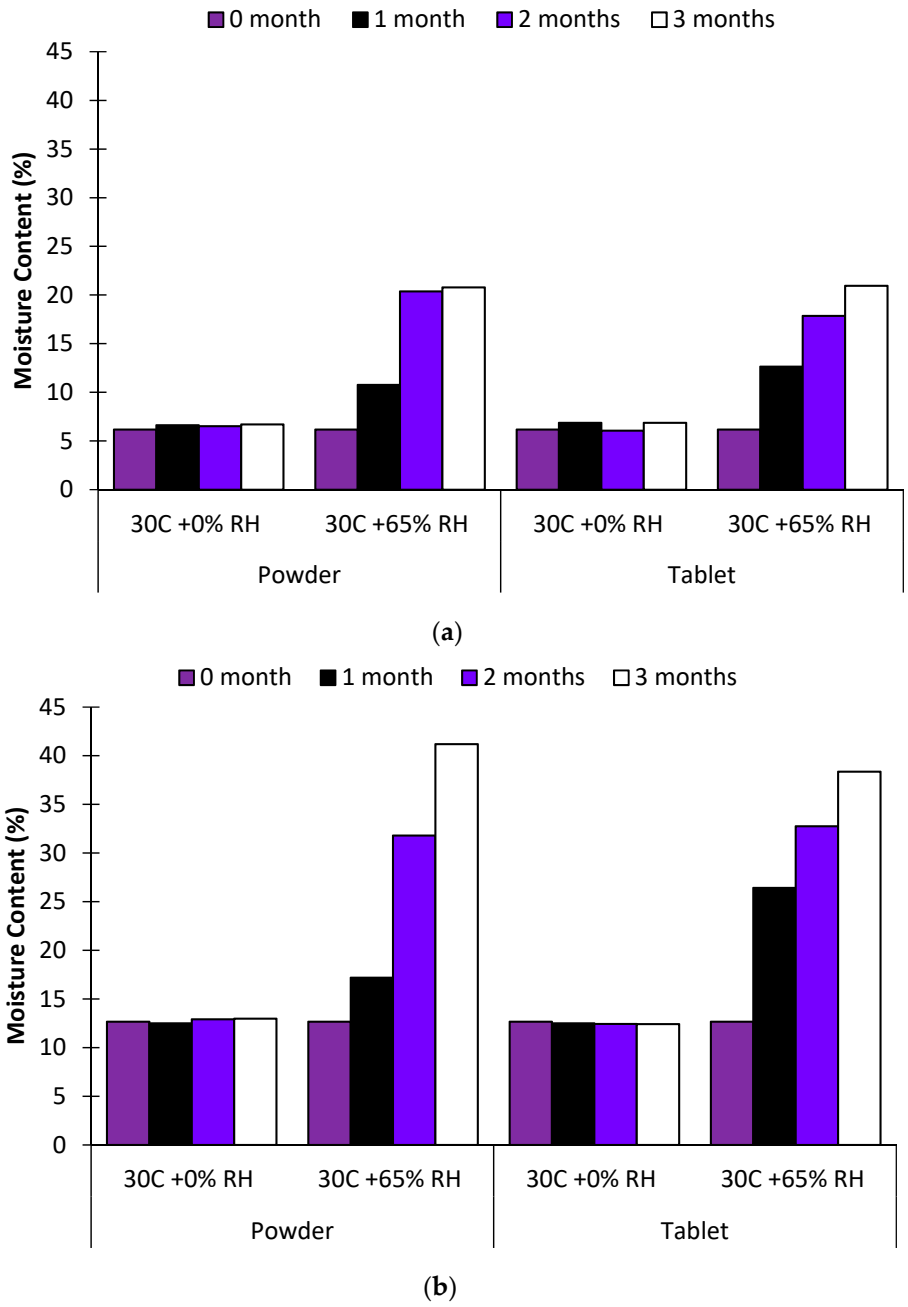


Figure 15. The moisture content of (**a**) PS21-S100 and (**b**) PS21-L100 formulations in powder and tablet forms at 30 °C and 0% RH and 30 °C and 65% RH. The moisture absorption in powders was slightly higher than that in tablets. The absorption pattern in both forms was similar. PS21-L100 underwent a significant increase in moisture content within three months of storage, while this was less significant for PS12-S100.

4. Discussion

This study has identified the critical quality attributes of the formulations and their associated process parameters [17,18]. The results show that the window of interest should be between 10 and 15 kN of tablet compression. These tablets are nominally 0.29 g and PFU/g of 1×10^9, giving a tablet dosage of 2.9×10^8 PFU, which is typically of the order used for phage therapy [19,20].

Dissolution tests showed that the phage was completely delivered within 3 h in the presence of SIF, which is compatible with in vivo models of the small intestine, in which the residence time is 3–4 h [21,22]. Despite significant tablet disintegration under the acidic

conditions of SGF, the fragments completely protected the phages and produced similar PFU values when subsequently released in SIF to tablets that had not been exposed to acidic conditions; this means that the requirement for tablets to remain intact within the stomach is not essential and, in this particular case, allows a formulation without the additional cost of an enteric coating to the tablet.

Early research with phages involved the intravenous administration of solutions [23], in which the protein structure of the phages was maintained in an aqueous solution. However, Grasmeijer et al. [24] discussed the main mechanisms of how sugars can stabilise phages in a solid system, immediately opening up the possibilities of oral [9] and inhalation [6] routes. Within an aqueous system, hydrogen bonding between the phage proteins and water molecules maintains the integrity of the protein structure; this "wet" system can be replaced by a "dry" system of amorphous solid sugar, in which the hydrogen bonds can either be formed with water molecules trapped within the system or with amorphous sugar molecules that follow the contours of the protein. These are known as the *water entrapment* and *water replacement* theories, respectively. Alternatively, the protein can be immobilised within the sugar matrix, preventing degradation through translational molecular movements; this is known as the *vitrification* theory.

In this study, spray-drying the phages with trehalose provided an opportunity to create an amorphous sugar system which allows these mechanisms to occur [24,25]. Analysis using differential scanning calorimetry (DSC) showed that this amorphous matrix existed, confirming earlier observations using similar formulations [13]. However, even when mixed with the Eudragit polymer, the system is metastable, hydrophilic and greatly influenced by the temperature and relative humidity of its surroundings. A transition to a more crystalline form through reactions with moisture will compromise phage viability as hydrogen bonds are broken and the sugar matrix modifies [26]. Extreme amounts of moisture pickup will also make the amorphous glassy structure change to a sticky or rubbery system; the storage tests clearly showed an inverse correlation between the phage count per gram and an increase in moisture content, although this degradation still occurred with no moisture increase [27].

A previous detailed analysis of trehalose indicated that an abrupt change in the glass transition temperature occurred at 50% RH [28]; although obtaining a reliable reading from a trehalose-polymer system is difficult, it can be inferred that adverse morphological changes occur in the particle structure at high relative humidity, leading to a degradation of the phages.

Storage under either fridge conditions of 4 °C or 20 °C is feasible for both formulations for up to a month, after which crystallisation of the amorphous structure begins to affect the phages. Storage at 30 °C results in a more rapid deterioration, even in the absence of humidity. These experiments indicate that the trehalose–Eudragit amorphous structure is metastable and will tend towards a crystalline structure even at a low temperature and relative humidity. Degradation occurred even in the absence of moisture uptake.

5. Conclusions

This study examined whether encapsulating phages in amorphous trehalose/Eudragit spray-dried particles would deal with the challenges of phage deactivation through contact with acidic conditions, high temperatures and moisture uptake; the intention was to create a solid dosage form that would be simple and cheap to produce, release the phages in the desired parts of the GI tract and have enough stability to be stored and administered in the absence of a cold supply chain. Including trehalose in the spray-dried formulation ensured that the phages were protected from the high temperature in the spray-drying process and also stabilised the phage structure during storage. Eudragit remained insoluble under acidic conditions, providing protection to the phages in the SGF before allowing their release in the more benign conditions of SIF. An aspect that requires further investigation is increasing the stability of the phages through formulation design, which currently start to degrade after 1 month.

Author Contributions: Conceptualization, M.C.L. and D.J.M.; methodology Z.R.Y., M.C.L. and D.J.M.; formal analysis, Z.R.Y. and D.J.M.; investigation, Z.R.Y.; resources, D.J.M. and M.C.L.; writing—original draft preparation, Z.R.Y.; writing—review and editing, M.C.L. and D.J.M.; supervision D.J.M. and Z.R.Y.; project administration, M.C.L. and D.J.M.; funding acquisition, M.C.L. and D.J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the UK Engineering and Physical Sciences Research Council grant number EP/M027341/1.

Data Availability Statement: The data is available on request from the corresponding author.

Acknowledgments: D.M. acknowledges funding from the UK Engineering and Physical Sciences Research Council (Grant No. EP/M027341/1), Tackling Antimicrobial Resistance: An Interdisciplinary Approach. Z.R.Y. acknowledges Loughborough-University-funded PhD studentship.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yen, C.; Shih, S.-M.; Tate, J.E.; Wu, F.-T.; Huang, Y.-C.; Parashar, U.D.; Hsiung, C.A. Intussusception-related Hospitalizations Among Infants Before and After Private Market Licensure of Rotavirus Vaccines in Taiwan, 2001–2013. *Pediatr. Infect. Dis. J.* **2017**, *36*, e252. [CrossRef]
2. Sulakvelidze, A.; Aladvidze, Z.; Morris, J.G. Bacteriophage Therapy. *Antimicrob. Agents Chemother.* **2001**, *45*, 649–659. [CrossRef] [PubMed]
3. Fair, R.J.; Tor, Y. Antibiotics and bacterial resistance in the 21st Century. *Perspect. Med. Chem.* **2014**, *6*, 25–64. [CrossRef] [PubMed]
4. Mokomane, M.; Kasvosve, I.; de Melo, E.; Pernica, J.M.; Goldfarb, D.M. The global problem of childhood diarrhoeal diseases: Emerging strategies in prevention and management. *Ther. Adv. Infect. Dis.* **2018**, *5*, 29–43. [CrossRef] [PubMed]
5. Aslam, B.; Wang, W.; Arshad, M.I.; Khurshid, M.; Muzammil, S.; Rasool, M.H.; Nisar, M.A.; Alvi, R.F.; Aslam, M.A.; Qamar, M.U.; et al. Antibiotic resistance—A rundown of a global crisis. *Infect. Drug Resist.* **2018**, *11*, 1645–1658. [CrossRef]
6. Chang, R.Y.K.; Wallin, M.; Kutter, E.; Morales, S.; Britton, W.; Li, J.; Chan, H.-K. Storage stability of inhalable phage powders containing lactose at ambient conditions. *Int. J. Pharm.* **2019**, *560*, 11–18. [CrossRef]
7. Chang, R.Y.K.; Kwok, P.C.L.; Khanal, D.; Morales, S.; Kutter, E.; Li, J.; Chan, H.-K. Inhalable bacteriophage powders: Glass transition temperature and bioactivity stabilization. *Bioeng. Transl. Med.* **2020**, *5*, e10159. [CrossRef]
8. Chang, R.Y.K.; Okamoto, Y.; Morales, S.; Kutter, E.; Chan, H.-K. Hydrogel formulations containing non-ionic polymers for topical delivery of bacteriophages. *Int. J. Pharm.* **2021**, *605*, 120850. [CrossRef]
9. Khanal, D.; Chang, R.Y.K.; Hick, C.; Morales, S.; Chan, H.-K. Enteric-coated bacteriophage tablets for oral administration against gastrointestinal infections. *Int. J. Pharm.* **2021**, *609*, 121206. [CrossRef]
10. Carrigy, N.B.; Liang, L.; Wanga, H.; Kariukic, S.; Nageld, T.E.; Connerton, I.F.; Vehring, R. Spray-dried anti-Campylobacter bacteriophage CP30A powder suitable for global distribution without cold chain infrastructure. *Int. J. Pharm.* **2019**, *569*, 118601. [CrossRef]
11. Malik, D.J.; Sokolov, I.J.; Vinner, G.K.; Mancuso, F.; Cinquerrui, S.; Vladisavljevic, G.T.; Clokie, M.R.J.; Garton, N.J.; Stapley, A.G.F.; Kirpichnikova, A. Formulation, Stabilisation and Encapsulation of Bacteriophage for Phage Therapy. *Adv. Colloid Interface Sci.* **2017**, *249*, 1–34. [CrossRef] [PubMed]
12. Malik, D.J.; Resch, G. Editorial: Manufacturing, Formulation and Delivery Issues for Phage Therapy to Become A Reality. *Front. Microbiol.* **2020**, *11*, 584137. [CrossRef]
13. Vinner, G.K.; Rezaie-Yazdi, Z.; Leppanen, M.; Stapley, A.G.F.; Leaper, M.C.; Malik, D.J. Microencapsulation of Salmonella-specific bacteriophage Felix O1 using spray-drying in a pH-responsive formulation and direct compression tableting of powders into a solid oral dosage form. *Pharmaceuticals* **2019**, *12*, 43. [CrossRef] [PubMed]
14. Tabare, E.; Dauchot, T.; Cochez, C.; Glonti, T.; Antoine, C.L.; Pirnay, J.-P.; Delcenserie, T.D.; Goole, J.; Eudragit, F.S. Microparticles Containing Bacteriophages, Prepared by Spray-Drying for Oral Administration. *Pharmaceuticals* **2019**, *12*, 1602. [CrossRef]
15. Vinner, G.K.; Malik, D.J. High precision microfluidic microencapsulation of bacteriophages for enteric delivery. *Res. Microbiol.* **2018**, *169*, 522–530. [CrossRef]
16. WHO. Annex 2 Stability Testing of Active Pharmaceutical Ingredients and Finished Pharmaceutical Products. *WHO Techn. Rep. Ser.* **2009**, *953*, 87–130.
17. Rathore, A.S. Roadmap for implementation of quality by design (QbD) for biotechnology products. *Trends Biotechnol.* **2009**, *27*, 546–553. [CrossRef]
18. Yu, L.X.; Amidon, G.; Khan, M.A.; Hoag, S.W.; Polli, J.; Raju, G.K.; Woodcock, J. Understanding pharmaceutical quality by design. *AAPS J.* **2014**, *16*, 771–783. [CrossRef]
19. Schooley, R.T.; Biswas, B.; Gill, J.J.; Hernandez-Morales, A.; Lancaster, J.; Lessor, L.; Barr, J.J.; Reed, S.L.; Rohwer, F.; Benler, S.; et al. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection. *Antimicrob. Agents Chemother.* **2017**, *61*, 110–128. [CrossRef]

20. Abedon, S. Chapter 1—Phage therapy pharmacology: Calculating phage dosing. In *Advances in Applied Microbiology*; Laskin, A.I., Sariaslani, S., Gadd, G.M., Eds.; Academic Press: Cambridge, MA, USA, 2011; pp. 1–40.
21. Yuen, K.-H. The transit of dosage forms through the small intestine. *Int. J. Pharm.* **2010**, *395*, 9–16. [CrossRef]
22. Karthikeyan, J.S.; Salvi, D.; Karwe, M.V. Modelling of fluid flow, carbohydrate digestion, and glucose absorption in human small intestine. *J. Food Eng.* **2021**, *292*, 110339. [CrossRef]
23. Syamov, R.M. Treatment and prophylaxis of cholera with bacteriophage. *Bull. WHO* **1963**, *28*, 361–367.
24. Grasmeijer, N.; Frijlink, H.W.; Hinrichs, W.L.J. Model to predict inhomogeneous protein–sugar distribution in powders prepared by spray drying. *J. Aerosol Sci.* **2016**, *101*, 22–33. [CrossRef]
25. Leung, S.S.; Parumasivam, T.; Gao, F.G.; Carrigy, N.B.; Vehring, R.; Finlay, W.H.; Morales, S.; Britton, W.J.; Kutter, E.; Chan, H.K. Production of Inhalation Phage Powders Using Spray Freeze Drying and Spray Drying Techniques for Treatment of Respiratory Infections. *Pharm. Res.* **2016**, *33*, 1486–1496. [CrossRef] [PubMed]
26. Roe, K.D.; Labuza, T.P. Glass transition and crystallization of amorphous trehalose-sucrose mixtures. *Int. J. Food Prop.* **2005**, *8*, 559–574. [CrossRef]
27. Vandenheuvel, D.; Meeus, J.; Lavigne, R.; Van Den Mooter, G. Instability of bacteriophages in spray-dried trehalose powders is caused by crystallization of the matrix. *Int. J. Pharm.* **2014**, *472*, 202–205. [CrossRef]
28. Bogdanova, E.; Fureby, A.M.; Kocherbitov, V. Hydration Enthalpies of Amorphous Sucrose, Trehalose and Maltodextrins and Their Relationship with Heat Capacities. *Phys. Chem. Chem. Phys.* **2021**, *23*, 14433–14448. [CrossRef]