HHS Public Access

Author manuscript

Int J Pharm. Author manuscript; available in PMC 2020 April 05.

Published in final edited form as:

Int J Pharm. 2019 April 05; 560: 11–18. doi:10.1016/j.ijpharm.2019.01.050.

Storage stability of inhalable phage powders containing lactose at ambient conditions

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Abstract

The aim of this study was to evaluate the storage stability of inhalable phage powders containing lactose and leucine as excipient. As an FDA-approved excipient for inhalation, lactose is preferred over other sugars. PEV phages active against antibiotic-resistant *Pseudomonas aeruginosa* was spray dried with lactose (55–90%) and leucine (45–10%). Produced powders were heat-sealed in an aluminium pouch at 15% relative humidity (RH) with subsequent storage at 20 °C/60% RH for 12 months. Lactose concentration in the powder positively influenced the phage stability over time. Formulation containing 90% lactose maintained the viability of PEV61 across the study, while ~1.2 $\log_{10}$ titer reduction was observed in formulations with less lactose. PEV20 was more prone to inactivation (1.7 $\log_{10}$ titer loss at 12-month) when lactose concentration in the particle was below 80%. The fine particle fraction (% wt. particles <5 μm in aerosol) of phage powders was 52 – 61% and remained the same after 12-month storage. The results demonstrate that spray dried PEV phage powders containing lactose and leucine are biologically and physically stable over long-term storage at ambient temperature. Furthermore, these spray dried phage powders were shown to be non-toxic to lung alveolar macrophage and epithelial cells *in vitro*.

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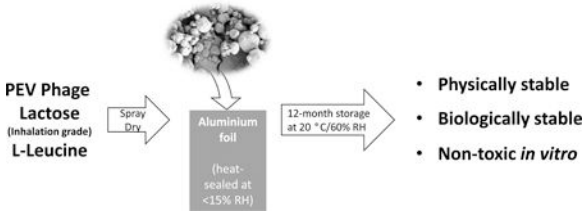
Conflict of interest

SM is an employee of AmpliPhi Biosciences, a publicly-traded company developing bacteriophage therapy products.

Graphical Abstract



Keywords

Bacteriophage (Phage); spray dried powders; lactose; storage stability; inhalation aerosol

1. Introduction

Infections caused by *Pseudomonas aeruginosa* is problematic in cystic fibrosis patients (Banerjee and Stableforth, 2000) with up to 95% at a risk of respiratory failure (Hansen et al., 2005). This Gram-negative pathogen deteriorates lung function by colonizing the lower parts of the respiratory tract. *P. aeruginosa* infections are difficult to treat due to its intrinsic and acquired resistance mechanisms to a wide range of antibiotics (Breidenstein et al., 2011). Antibiotic-resistant strains cause a three-fold higher rate of mortality and a nine-fold higher rate of further complications (Giamarellou, 2002). Bacteriophage (phage) therapy is a promising alternative option for combating infections caused by antibiotic-resistant strains (Parisien et al., 2008). Nebulization has been the method of choice for phage delivery to the lung. Depending on the type of nebulizer used, phages can be delivered as inhalable aerosol droplets with minimal titer loss (Astudillo et al., 2018; Carrigy et al., 2017; Golshahi et al., 2008). Nebulized phage aerosols have been successfully used in clinics for treating respiratory infections where antibiotic treatment had failed (Kutateladze and Adamia, 2008; Kvachadze et al., 2011).

Dry powder formulation offers advantages such as ease of administration, storage and transport. Compared to nebulizers, dry powder inhalers (DPI) are small, portable and can be operated without a power source with shorter treatment times. Moreover, routine cleaning and disinfection are not necessary for DPIs (Geller, 2008). Dry powders can be stored at ambient condition, which eliminates the need for cold chains thereby reducing the operating cost (Chew and Chan, 2002). Spray drying is commonly used for producing phage powders for pulmonary delivery. Inhalable spray dried phage powders can be produced with negligible titer loss (< 1 $\log_{10}$) (Chang et al., 2017; Leung et al., 2016; Vandenheuvel et al., 2013) and remain efficacious *in vivo* (Agarwal et al., 2018; Chang et al., 2018).

The stability of phages in dry powder depends on the phage type, formulation excipients and the preparation method (Ackermann et al., 2004; Franks, 1998). Ackermann *et al.* reported that phages remained viable after lyophilisation with 50% glycerol, even after 20 years of storage at 4 °C under vacuum (Ackermann et al., 2004). Merabishvili *et al.* demonstrated stability of *Staphylococcus aureus* phage ISP after lyophilisation with 0.5 M sucrose or 0.5 M trehalose over 27-month storage period (Merabishvili et al., 2013). Vandenheuvel *et al.*

assessed the storage stability of *P. aeruginosa* phage LUZ19 and *S. aureus* phage Romulus after spray drying with trehalose (Vandenheuvel et al., 2014). LUZ19 remained stable after 12-month storage at 4 °C/0% relative humidity (RH) and lost only 1 $\log_{10}$ titer at 54% RH, whereas Romulus lost 2 and 7 $\log_{10}$ units at the respective storage conditions.

Leung *et al.* assessed the storage stability of spray dried powders of *P. aeruginosa* phage PEV2 (Leung et al., 2017). Phage PEV2 was spray dried with trehalose (0 – 80%), mannitol (0 – 80%) and leucine (20%), followed by storage at 4 °C and 0%, 22% or 60% RH. Phage stability and *in vitro* aerosol performance were examined after 0, 1, 3 and 12 months of storage. In the presence of trehalose, phages remained viable at 0% and 22% RH after 12-month storage, with ~10^5 plaque forming units (pfu) of phages in the particles below 5 μm in aerodynamic diameter. Storage at 60% RH was detrimental for phage stability and complete inactivation was observed after 3 months. PEV2 was most stable in spray dried powders containing >40% trehalose at 4 °C and <22% RH. A more recent study examined storage stability of spray dried PEV2 (Podovirus) and PEV40 (Myovirus) (Leung et al., 2018). Phages were spray dried with trehalose and leucine, followed by storage at 4 °C or 20 °C under vacuum. Both phage types remained stable with <1 $\log_{10}$ titer reduction after 12 months of storage at 4 °C or 20 °C with vacuum packaging.

Previously, we used design of experiments to identify key excipients necessary for stabilization and aerosolization of spray dried PEV phages (PEV1, PEV20 and PEV61) (Chang et al., 2017). Eight formulations containing lactose and leucine preserved the phage bioactivity in spray dried powders with a high fine particle fraction of over 50%. In this study, lactose provided superior phage protection to trehalose. Use of lactose over trehalose provides significant advantage as lactose is an US Food and Drug Administration (FDA)-approved excipient for inhalation products. For inhalable phage powders to become a viable commercial product, biological stability of phages and physical stability of powders are essential. Furthermore, storage stability assessment is vital for determining the anticipated shelf-life. The aim of this study was to examine the storage stability, including biological and physicochemical, of eight phage formulations over 12 months.

2. Methods

2.1 Materials

Three pseudomonas lytic phages PEV1, PEV20 and PEV61 were obtained from AmpliPhi Biosciences AU at a high titer of 10^{10} pfu/mL. Phages were stored in a phosphate buffered saline (PBS, 0.01 M phosphate buffer, 0.137 M NaCl and 0.0027 M KCl) with pH 7.4. PEV phages were isolated from the sewage treatment plant in Olympia, WA, USA by the Kutter Lab (Evergreen Phage Lab) and *P. aeruginosa* dog-ear strain PAV237 was used to amplify the phages. Lactose monohydrate (DFE Pharma, Goch, Germany) and L-leucine (Sigma-Aldrich) were used as excipients. These excipients can protect phages from stresses of spray drying with minimal titer reduction (Chang et al., 2017). Table 1 shows the composition of eight formulations prepared in this study.

2.2 Phage powder preparation

Spray dried phage powders were prepared as per our previous study (Chang et al., 2017). Briefly, the liquid feed composed of 2 mL of phage suspension (10^{10} pfu/mL) and 198 mL of excipient solution (pH 7.4) at a total solid content of 25 mg/mL. The phage viability in the feed solution before spray drying was confirmed using a standard plaque assay. The mixtures were spray dried using a Büchi 290 spray dryer (Buchi Labortechnik AG, Flawil, Switzerland) coupled with a conventional two-fluid nozzle for atomization with an open loop setting. Spray drying conditions were as follows: aspiration rate of 35 m^3/h, feed rate of 2 mL/min, atomizing airflow of 742 L/h, inlet temperature of 60 °C and outlet temperature of 40–41 °C. The produced powders were aliquoted into scintillation vials and heat-sealed in an aluminium pouch Phage powder aliquoting and heat-sealing was done inside an acrylic box maintained at 15 ± 2 % RH. Aluminium pouches were stored at 20 °C/60% RH for 12 months. Phage stability and powder physicochemical properties were examined after 0, 1, 6 and 12 months of storage.

2.3 Plaque assay

Phage titer in the spray dried powders was determined after reconstitution in PBS. Reconstituted phage lysate was serially diluted by adding 20 μL samples to 180 μL of PBS. A volume of (200 μL) *P. aeruginosa* dog-ear strain PAV237 containing 2×10^8 colony forming units at stationary phase was mixed with 5 mL of 0.4% nutrient broth top agar. The mixture was overlaid onto a 1.5% nutrient agar plate. Then, 10 μL of diluted phage suspension was dropped on top of the top agar plate, air dried and then incubated at 37 °C for 18 h. The assay was independently conducted three times. Student's t test was used to examine the statistical significance of the data. The null hypothesis was rejected if the p value was <0.05.

2.4 Scanning electron microscopy

Particle morphology of the spray dried phage powders was examined using scanning electron microscopy (SEM) (Zeiss Ultra Plus, Carl Zeiss NTS GmbHm Oberkochen, Germany). The samples were mounted on a carbon tape and sputter coated with 15 nm of gold using a K550X sputter coater (Quorum Emitech, Kent, UK) before imaging.

2.5 Particle size distribution

Particle size distribution of phage powders was measured by laser diffraction (Mastersizer 2000, Malvern Instruments Ltd., UK). The powders were loaded onto Scirocco 2000 dry powder module (Malvern Instruments, UK) and dispersed through the measurement window with compressed air at 4.0 bars. Size distribution was expressed by volumetric diameters (D_{10}, D_{50}, and D_{90}) and span ($(D_{90}-D_{10})/D_{50}$). All measurements were done in triplicate.

2.6 Dynamic vapour sorption

Moisture sorption profile of the phage powders was analysed using a dynamic vapour sorption (DVS) instrument (DVS-Instrinsic, Surface Measurement Systems, London, UK). Each sample (10 mg) was exposed to a moisture ramping cycle of 0 to 60% RH at a step

increase of 10%. Criterion for equilibrium moisture content at each RH was defined as less than dm/dt of 0.02%/min.

2.7 Thermal gravimetric analysis

Thermal gravimetric analysis (TGA) instrument (Mettler Toledo, Greifensee, Switzerland) was used to analyse the thermal property of phage powders. Each sample (5 ± 1 mg) was weighed in to a crucible and heated from 30 to 150 °C at a rate of 10 °C/min with dynamic nitrogen flow. Data analysis was conducted using STARe software (V.9.0x; Mettler Toledo, Greifensee, Switzerland). The assay was independently conducted twice.

2.8 Differential scanning calorimeter

Each sample (5 ± 1 mg) was weighed into an aluminium crucible and then crimped to a perforated lid. The crucible was placed inside differential scanning calorimeter (DSC) instrument (Mettler Toledo, Greifensee, Switzerland) and heated from 22 to 150 °C at a rate of 10 °C/min with dynamic nitrogen flow. The assay was independently conducted twice.

2.9 In vitro cell viability assay

Toxicity of the spray dried phage powder formulations was assessed *in vitro* using resazurin cell viability assay. Three cell lines, including A549, HEK239 and THP-1 (10^5 cells) were exposed to phage formulations at 10, 100 and 1000 mg/L for 24 h. Cells were incubated with resazurin at 37 °C and 5% CO_2 for 2 h. The fluorescence was measured using a plate reader at excitation and emission wavelengths of 530 nm and 590 nm, respectively (FLUOstar Omega, BMG LABTECH GmbH, Ortenberg, Germany)

2.10 In vitro aerosol performance

In vitro aerosol performance of the phage powders was assessed as per our previous study (Chang et al., 2017) after 0, 1, 6 and 12 months of storage. Briefly, each sample (30 mg) was loaded to a size 3 hydroxypropyl methylcellulose capsule (Capsugel, NSW, Australia) in a humidity controlled Perspex box (<17% RH) and dispersed using an Osmohaler™ at 100 L/min for 2.4 s. The inhaler was connected to a stainless steel throat (United States Pharmacopeia throat) and multi-stage liquid impinger (MSLI). Ultrapure water (18.2 MΩ) was used as collecting solvent to determine deposition profiles of lactose. Cut-off diameters of the MSLI stages 1 – 4 at 100 L/min are 10.1, 5.3, 2.4 and 1.32 μm, respectively. Fine particle fraction (FPF) was defined as the mass fraction of particles ≤5.0 μm with respect to the loaded dose. Experiments were done in triplicate. Statistical significance of the data was examined using Student's t test. The null hypothesis was rejected if the p value was <0.05.

2.11 High-performance liquid chromatography chemical assay

The deposition of lactose in the inhaler, capsule, adaptor, throat and each part of the MSLI was determined using a high-performance liquid chromatography (HPLC) system with refractive index detection (Model LC-20; Shimadzu, Japan). The HPLC system consisted of a SIL-20A HT auto-sampler, CBM-20A controller, LC-20AT pump, RID-10A RI detector, Agilent Hi-Plex Ca^{2+} Ligand Exchange Columns (300 × 7.7 mm, 8 μm; Phenomenex, Torrance, CA), and LC Solution software. The chromatographic conditions were as follows:

mobile phase ultra-pure water; flow rate 0.6 mL/min; injection volume 50 μL; oven temperature 85 °C.

3. Results and Discussions

3.1 Particle morphology

Spray dried phage powders contained mostly spherical particles of less than 2 μm (Fig. 1). Similar to our previous study (Chang et al., 2017), decreased lactose and increased leucine content led to formation of irregular platelet-shaped particles with rough surfaces. Formulations containing the same lactose and leucine content had the same particle morphology irrespective of the phage used. PEV20 and PEV61 spray dried with 80% lactose and 20% leucine both formed spherical particles with smooth surfaces. Phage powders containing 70% lactose all formed corrugated particles with rough surfaces. No noticeable changes were observed throughout 12 months of storage.

3.2 Particle size distribution

The volume median diameters and span values of the phage powders were 1.8–2.1 μm and 1.4–1.9, respectively (Table 2). The results suggest that these powders are suitable for inhalation delivery to the lung. The size distribution did not vary notably with the excipient content nor the phage type, and practically no change was observed in all the phage formulations after 12-month storage.

3.3 Thermal analysis

The phage powder formulations showed a glass transition temperature of 60–63 °C (Table 3), reflecting the amorphous nature of the phage powder. XRD data confirmed the amorphous nature, showing a halo diffraction pattern without sharp diffraction peaks (Fig. 2). The crystallinity of phage powders was increased with leucine content, as shown by sharper additional peaks. These phage powders had a water content of 1.5–3.2 wt % (Table 3). After 12-month storage, the glass transition temperature increased by 4–9 °C. TGA data showed that the water content remained at similar level, except the phage powder containing 90% lactose and 10% leucine (which showed a reduction from 4.5 to 2.6 wt %). Amorphous glassy state is thermodynamically unstable. During storage, the molecular structures relax toward the equilibrium state, thereby decreasing the molecular mobility and free volume in the glassy state (Badii et al., 2005; Haque et al., 2006). Glass transition temperature increases and the relaxation endotherm shifts to higher temperatures with aging time (Haque et al., 2006; Lammert et al., 1999). Our results showed that during long-term storage a more rigid glass might have formed, which explains the increase in glass transition temperature.

3.4 Moisture sorption profile

All the phage powders showed a sorption profile with the characteristic of a re-crystallization event. That is, powders initially absorbed moisture with mass increase, then at a certain threshold RH re-crystallisation caused mass decrease as water was expelled from the crystal lattice (Fig. 3.). The overall mass decreased within 16–20 min of exposure to 60% RH, where amorphous content transited to crystalline form releasing excess water. The water sorption capacity was increased with the lactose content (Fig. 3). Hydrophobic leucine

forms a crystalline shell on the surface of spray dried powders and acts as a shield against moisture-induced powder degradation (Li et al., 2016). However, even 45% leucine was unable to prevent re-crystallization at 60% RH. These results highlight the importance of low RH when handling and storing spray dried phage powders (Leung et al., 2017; Vandenheuvel et al., 2014). Leung *et al.* reported recrystallization of phage powders containing trehalose and leucine at 50% RH (Leung et al., 2017). This suggests that lactose and leucine combination is more resilient to moisture-induced powder degradation than trehalose-leucine combination.

3.5 In vitro aerosol performance

The fine particle fraction (FPF, % wt. particles <5 μm in aerosol) ranged 52–61% for all phage powders (Fig. 4). No notable difference or trend in FPF was observed among different formulation compositions. After 6-month storage at 60% RH, the FPF slightly dropped to 48–56%, but these changes were not statistically significant. No further deterioration in aerosol performance was observed at 12-month.

3.6 In vitro toxicity of phage formulations

Lactose is well-recognized as a safe pharmaceutical excipient for inhalation products with no reported adverse effects to the lung (Baldrick and Bamford, 1997). Although leucine is yet to be approved for inhalation delivery, it is unlikely to constitute any significant toxicological hazard to human. More than 94% of human epithelial (A549 and HEK239) and macrophage (THP-1) cell lines survived after 24 h exposure to the spray dried phage powders at 10, 100 and 1,000 mg/L (Table 4). Cell survival was not concentration-dependent nor formulation composition-dependent. These results demonstrated the feasibility of producing safe spray dried phage powders in a laboratory setting.

3.7 Phage stability

Phages PEV1, PEV20 and PEV61 can infect and kill clinical and antibiotic-resistant *P. aeruginosa* strains (Chang et al., 2017). All the powder formulations had ~0.5 $\log_{10}$ titer reduction relative to the liquid feed stock (Table 1), confirming the stability of PEV phages in the spray drying process. Although the mechanism of phage stabilization in solid state is unclear, water substitution and/or vitrification are likely to be playing an important role (Chang et al., 2005; Grasmeijer et al., 2013).

Phages titer was largely retained for each of the formulations after 1-month storage (Fig. 5). PEV61 phage powder containing 90% lactose remained biologically stable with no further titer loss at 12-month assessment. On the other hand, those containing 70% and 80% lactose resulted in 0.5 $\log_{10}$ and 0.4 $\log_{10}$ titer loss, respectively. Phage powders containing an even less lactose content of 55% resulted in 0.7 $\log_{10}$ titer reduction at 6-month, followed by 1.2 $\log_{10}$ at 12-month assessment. Similar results were observed for phages PEV1 and PEV20. PEV1 powder containing 70% lactose lost 0.4 $\log_{10}$ in titer at 6-month with no further loss at 12-month assessment point. These results suggest that a high lactose content, preferably >80%, is required to stabilize the phages in solid state over long-term storage. Likewise, PEV20 powders with 55–80% lactose remained relatively stable with 0.2–0.4 $\log_{10}$ titer reduction at 6-month. However, phage stability was only preserved in powders containing

80% lactose at 12-month. Those containing <70% lactose resulted in up to 1.7 $\log_{10}$ titer reduction. Therefore, compared to the other two phages, PEV20 was more prone to inactivation over longer storage time when the lactose content in the formulation was below 80%. Long-term storage stability of phages has been reported to depend on different phages types and formulation compositions. Leung *et al.* reported that spray dried PEV2 powders containing 70% trehalose/30% leucine remained biologically stable after 12-month storage at 20 °C/0% RH, whereas PEV40 powders lost 0.6 $\log_{10}$ in titer (Leung et al., 2018). Furthermore, phage stability was compromised (0.8–0.9 $\log_{10}$ loss) when the trehalose content was lowered to 60%.

Dry powder phage formulations for respiratory delivery requires both physical stability of powders and biological viability of phages over storage. Leucine is often used to enhance the dispersibility of powders and minimize any moisture-induced powder degradation (Li et al., 2016). It has surfactant-like properties and forms an outer shell on the surface of spray dried particles (Leung et al., 2018). Therefore, leucine may help improve aerosol performance of the powders and also storage stability of phages (Mensink et al., 2017). Compared to smaller sugar molecules, phages have a relatively large size which will prevent them from diffusing away from the droplet surface during the drying process (Adler et al., 2000). Hence, surfactants could potentially protect phages against interfacial stresses (Mensink et al., 2017). This study demonstrated that lactose not only can protect phages from potential shear and thermal stresses during spray drying, but also stabilize them during storage. Phage bioactivity was well-protected over a 12-month period in the presence of high lactose content. Use of lactose as a phage stabilizer for pulmonary application offers significant advantage as it is an approved excipient for inhalation products.

4. Conclusions

We assessed the storage stability of eight spray dried phage powders containing lactose and leucine. Our results indicate that these two excipients provide both biological and physical stability after 12-month storage at 20 °C/60% RH when packaged under dry condition. Phage viability was largely dependent on lactose concentration, with higher concentration providing better phage protection. To maintain the physical stability of powders during long term storage, a small amount of leucine (10%) was found to be sufficient. Furthermore, the phage powders were non-toxic to epithelial and macrophage cells *in vitro*.

Acknowledgements

Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number R33 AI121627 (H.-K.C. and J.L.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. H.-K.C. is also supported by the Australian National Health and Medical Research Council (NHMRC, APP1140617).

Abbreviations:

DPI	Dry powder inhalers
RH	relative humidity

pfu	plaque forming units
FDA	Food and Drug Administration
PBS	phosphate buffered saline
DVS	dynamic vapour sorption
TGA	thermal gravimetric analysis
DSC	differential scanning calorimeter
MSLI	multi-stage liquid impinge
FPF	fine particle fraction
HPLC	high-performance liquid chromatography

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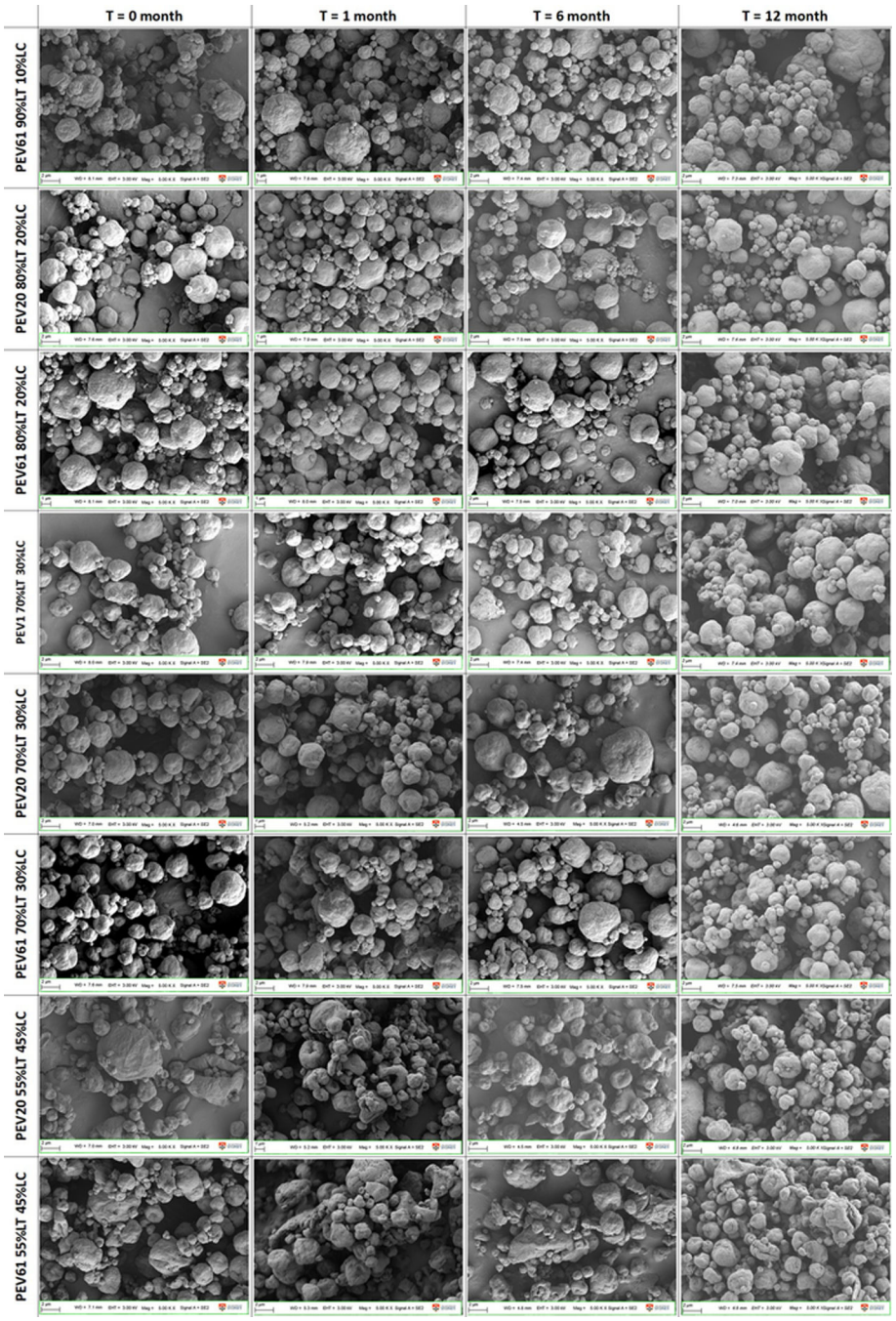

Fig. 1.

SEM images of spray dried phage powders in sealed aluminium pouches before and after 1, 6 and 12-month storage at 20 °C/60% RH. The phage powders were heat-sealed in aluminium pouches inside an acrylic box maintained at 15% RH.

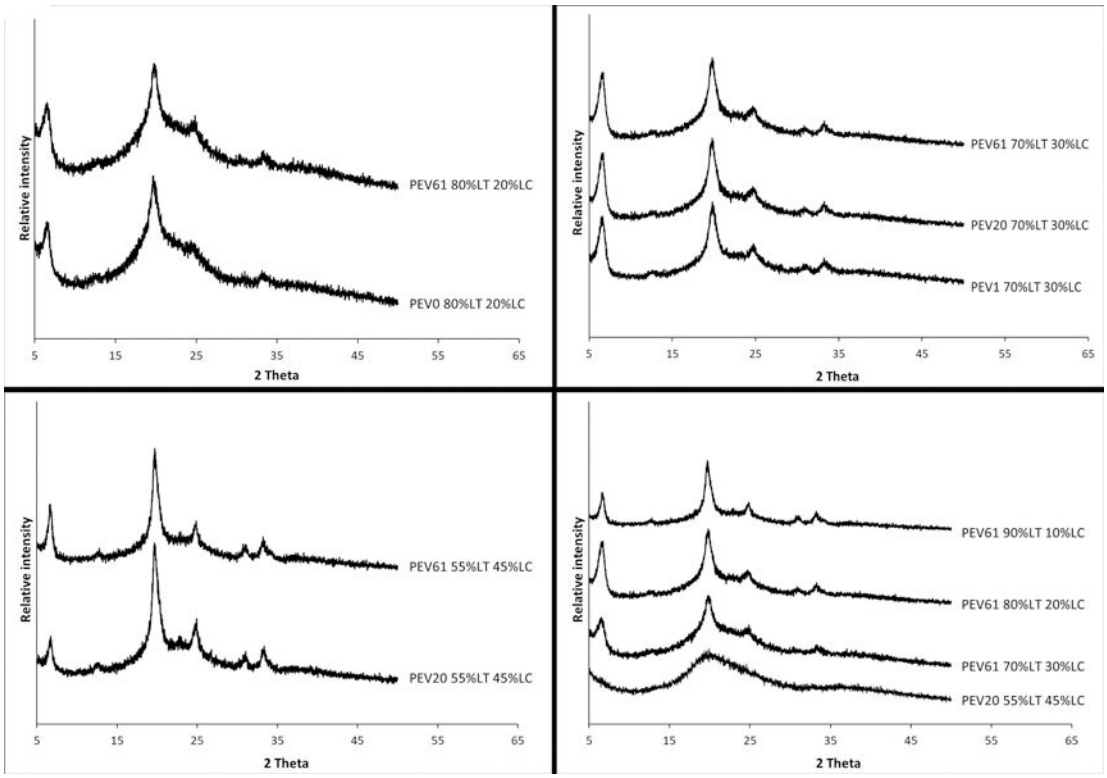

Fig. 2.
X-ray powder diffraction patterns of spray dried phage powders.

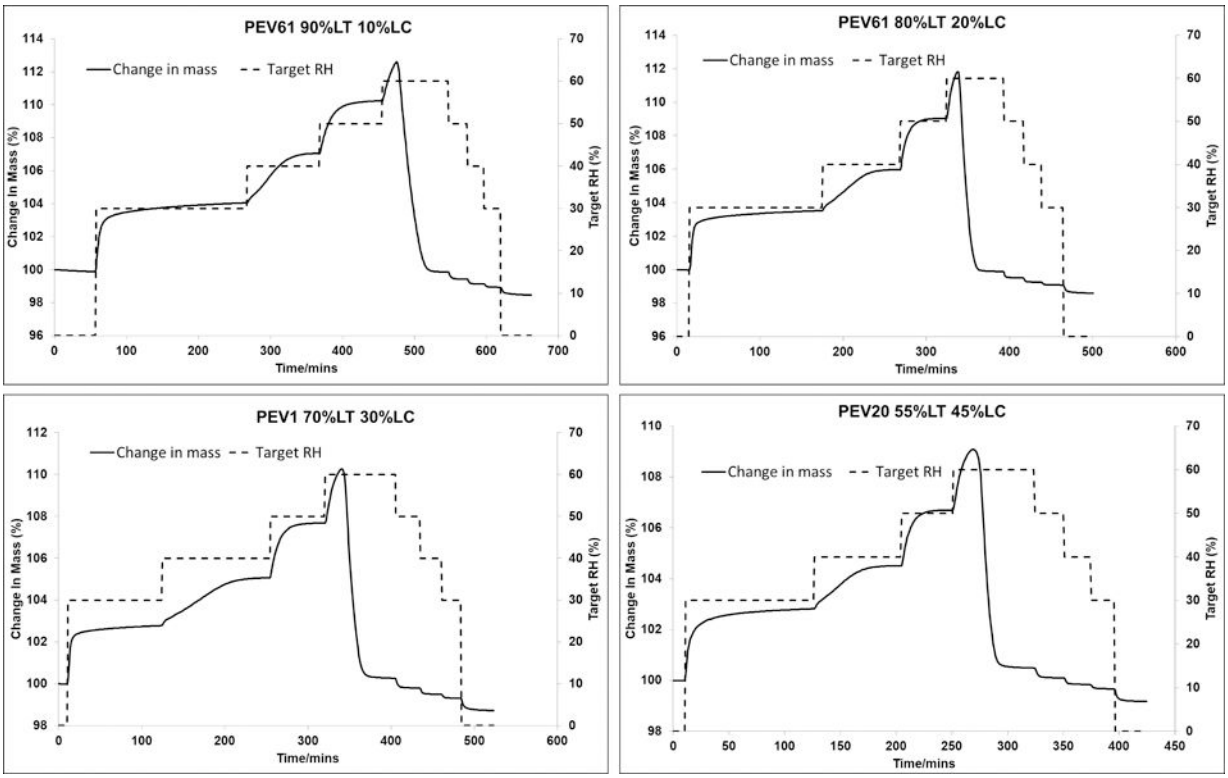

Fig. 3.

Moisture sorption kinetic profiles for representative phage formulations, including PEV61 90%LC 10%LC, PEV61 80%LT 20%LC, PEV1 70%LT 30%LC and PEV20 55%LT 45%LC.

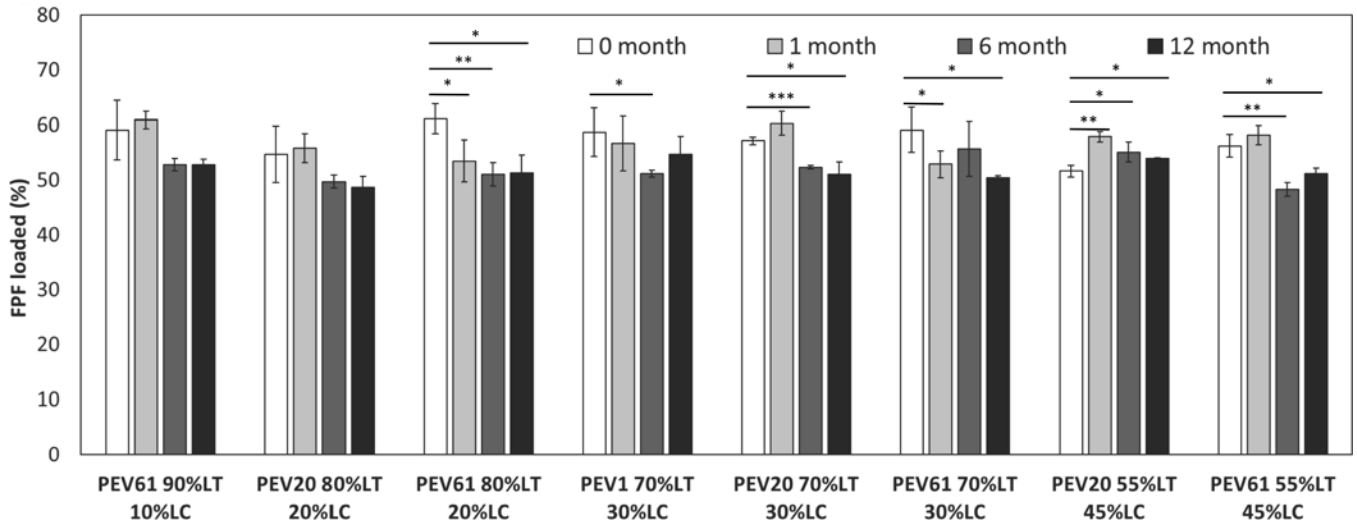

Fig. 4.

Fine particle fraction of aerosolized phage formulations after 0, 1, 6 and 12-month storage. All formulations were dispersed using an Osmohaler™ at 100 L/min for 2.4 s. Data presented as mean ± standard deviation ($n = 3$). Student's t test was used for statistical analysis. *, $P < 0.05$; **, $P < 0.001$. NOTE: FPF, fine particle fraction (< 5 μm in aerosol).

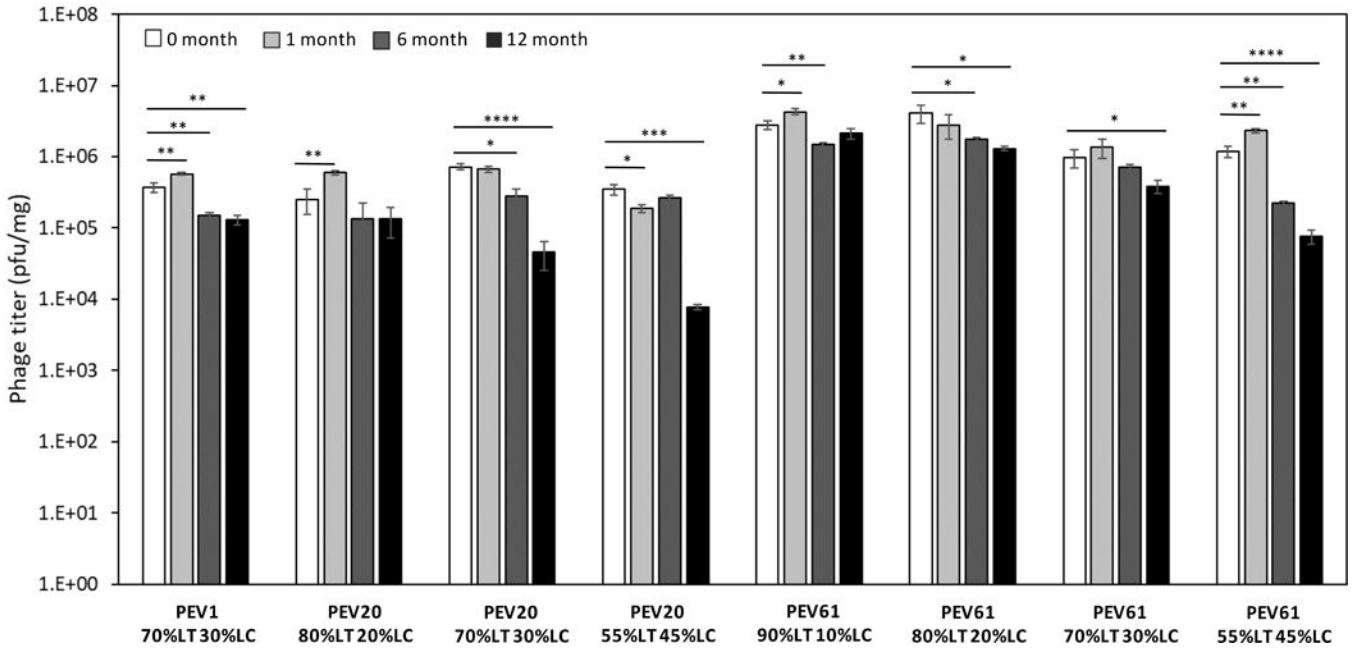

Fig. 5.

Phage titer in spray dried powders in sealed aluminium pouches after 0, 1, 6, 12-month storage at 20 °C/60% RH. Student's t test was used for statistical analysis. *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$. The phage powders were heat-sealed in aluminium pouches inside an acrylic box maintained at 15% RH.

Table 1.

Formulation compositions and loss of phage bioactivity after spray drying.

Formulation name	Phage	Contents % (w/w)		Log_{10} titer loss after spray drying
		Lactose	Leucine	
PEV61 90%LT 10%LC	PEV61	90	10	0.3
PEV20 80%LT 20%LC	PEV20	80	20	0.2
PEV61 80%LT 20%LC	PEV61	80	20	0.2
PEV1 70%LT 30%LC	PEV1	70	30	0.2
PEV20 70%LT 30%LC	PEV20	70	30	0.5
PEV61 70%LT 30%LC	PEV61	70	30	0.3
PEV20 55%LT 45%LC	PEV20	55	45	0.6
PEV61 55%LT 45%LC	PEV61	55	45	0.4

Table 2

Particle size distributions of phage powders spray dried before and after a 12-month storage at 20 °C/15% RH.

Formulation	T = 0 month				T = 12 month			
	D10 (μm)	D50 (μm)	D90 (μm)	Span	D10 (μm)	D50 (μm)	D90 (μm)	Span
PEV61 90%LT 10%LC	0.75 ± 0.00	1.90 ± 0.00	3.86 ± 0.03	1.63 ± 0.02	0.69 ± 0.11	1.81 ± 0.02	3.74 ± 0.09	1.69 ± 0.06
PEV20 80%LT 20%LC	0.76 ± 0.01	1.96 ± 0.01	4.09 ± 0.28	1.70 ± 0.14	0.76 ± 0.02	1.83 ± 0.08	3.70 ± 0.13	1.61 ± 0.02
PEV61 80%LT 20%LC	0.75 ± 0.01	1.85 ± 0.05	3.83 ± 0.09	1.63 ± 0.01	0.79 ± 0.08	1.76 ± 0.10	3.45 ± 0.43	1.50 ± 0.21
PEV1 70%LT 30%LC	0.79 ± 0.00	1.80 ± 0.03	3.70 ± 0.05	1.58 ± 0.01	0.78 ± 0.02	1.86 ± 0.04	3.68 ± 0.06	1.56 ± 0.02
PEV20 70%LT 30%LC	0.68 ± 0.09	1.77 ± 0.07	3.70 ± 0.11	1.67 ± 0.07	0.84 ± 0.05	1.76 ± 0.20	3.51 ± 0.54	1.44 ± 0.19
PEV61 70%LT 30%LC	0.79 ± 0.01	1.90 ± 0.03	3.80 ± 0.05	1.85 ± 0.00	0.74 ± 0.03	1.77 ± 0.05	3.50 ± 0.07	1.60 ± 0.01
PEV20 55%LT 45%LC	0.84 ± 0.04	1.87 ± 0.03	3.76 ± 0.05	1.56 ± 0.04	0.86 ± 0.08	1.76 ± 0.18	3.25 ± 0.52	1.37 ± 0.17
PEV61 55%LT 45%LC	0.81 ± 0.09	2.05 ± 0.13	4.23 ± 0.12	1.67 ± 0.12	0.91 ± 0.11	2.11 ± 0.12	4.47 ± 0.56	1.67 ± 0.23

D50 represents a cumulative 50% point of diameter (D10 and D90 represents a cumulative 10% and 90% point of diameters, respectively). D50 values are in bold as they are also known as “average particle size”.

Table 3.

Residual moisture content and glass transition temperature (T*g*) of spray dried phage formulations.

Formulation	Moisture content (wt %)		Tg (°C)	
	0 month	12 month	0 month	12 month
PEV61 90%LT 10%LC	4.5 ± 0.4	2.6 ± 0.2	60.0 ± 2.0	66.1 ± 1.1
PEV20 80%LT 20%LC	2.8 ± 0.7	2.9 ± 0.3	62.3 ± 1.1	65.7 ± 0.6
PEV61 80%LT 20%LC	2.7 ± 0.1	2.7 ± 0.2	62.0 ± 1.8	67.2 ± 1.6
PEV1 70%LT 30%LC	2.2 ± 0.6	2.2 ± 0.1	61.5 ± 1.6	67.3 ± 0.7
PEV20 70%LT 30%LC	2.5 ± 0.8	2.4 ± 0.1	62.7 ± 0.2	69.2 ± 0.6
PEV61 70%LT 30%LC	2.5 ± 0.1	2.8 ± 0.3	60.5 ± 1.1	70.1 ± 0.4
PEV20 55%LT 45%LC	1.5 ± 0.7	1.4 ± 0.3	63.2 ± 2.7	66.9 ± 0.6
PEV61 55%LT 45%LC	1.9 ± 0.5	1.2 ± 0.2	61.6 ± 1.9	66.6 ± 0.3

Table 4.

In vitro cell viability assay of A549, HEK293 and THP-1 cells after 24 h exposure to phage formulations at 10, 100 and 1000 mg/L.

Formulation Name	A549 cell survival (%)			HEK293 cell survival (%)			THP-1 cell survival (%)		
	10 mg/L	100 mg/L	1000 mg/L	10 mg/L	100 mg/L	1000 mg/L	10 mg/L	100 mg/L	1000 mg/L
PEV61 90%LT 10%LC	96.3 ± 0.6	96.7 ± 0.7	96.6 ± 0.5	98.9 ± 0.6	98.8 ± 4.2	100.0 ± 2.1	96.3 ± 2.8	96.0 ± 1.3	97.1 ± 2.5
PEV20 80%LT 20%LC	95.4 ± 0.8	96.0 ± 1.2	98.4 ± 1.6	97.4 ± 1.4	96.5 ± 3.2	97.6 ± 6.5	96.5 ± 2.0	98.9 ± 2.2	98.0 ± 2.9
PEV61 80%LT 20%LC	94.7 ± 1.8	94.8 ± 3.2	98.0 ± 1.3	95.0 ± 0.6	94.2 ± 7.9	94.2 ± 1.1	97.7 ± 0.9	95.0 ± 0.8	98.8 ± 0.7
PEV1 70%LT 30%LC	96.9 ± 3.2	97.4 ± 1.3	98.0 ± 1.2	99.0 ± 3.0	96.0 ± 1.8	95.5 ± 0.4	99.0 ± 3.0	97.3 ± 2.0	96.0 ± 2.8
PEV20 70%LT 30%LC	95.5 ± 3.2	92.5 ± 3.9	97.0 ± 1.5	95.0 ± 5.5	92.1 ± 2.1	96.2 ± 7.6	97.4 ± 3.3	96.1 ± 7.1	95.5 ± 6.0
PEV61 70%LT 30%LC	96.1 ± 1.8	96.7 ± 0.3	96.9 ± 1.2	99.3 ± 2.3	95.1 ± 1.6	94.8 ± 1.0	97.1 ± 1.6	95.1 ± 1.6	96.8 ± 0.8
PEV20 55%LT 45%LC	97.9 ± 3.0	95.4 ± 2.4	98.0 ± 2.2	94.7 ± 5.5	94.7 ± 1.7	94.0 ± 2.0	97.5 ± 0.2	98.8 ± 2.4	94.5 ± 3.9
PEV61 55%LT 45%LC	96.5 ± 2.4	96.0 ± 1.4	96.9 ± 1.8	96.9 ± 2.4	96.8 ± 2.4	96.3 ± 1.3	95.8 ± 3.1	97.7 ± 2.1	99.0 ± 2.5