



## Research paper

## The effect of formulation on spray dried Sabin inactivated polio vaccine

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## ABSTRACT

The objective of this study was to develop a stable spray dried formulation, containing the three serotypes of Sabin inactivated polio vaccine (sIPV), aiming for minimal loss of native conformation (D-antigen) during drying and subsequent storage. The influence of atomization and drying stress during spray drying on trivalent sIPV was investigated. This was followed by excipient screening, in which monovalent sIPV was formulated and spray dried. Excipient combinations and concentrations were tailored to maximize both the antigen recovery of respective sIPV serotypes after spray drying and storage ( $T = 40\text{ }^{\circ}\text{C}$  and  $t = 7$  days). Furthermore, a fractional factorial design was developed around the most promising formulations to elucidate the contribution of each excipient in stabilizing D-antigen during drying. Serotype 1 and 2 could be dried with 98% and 97% recovery, respectively. When subsequently stored at  $40\text{ }^{\circ}\text{C}$  for 7 days, the D-antigenicity of serotype 1 was fully retained. For serotype 2 the D-antigenicity dropped to 71%. Serotype 3 was more challenging to stabilize and a recovery of 56% was attained after drying, followed by a further loss of 37% after storage at  $40\text{ }^{\circ}\text{C}$  for 7 days. Further studies using a design of experiments approach demonstrated that trehalose/monosodium glutamate and maltodextrin/arginine combinations were crucial for stabilizing serotype 1 and 2, respectively. For sIPV serotype 3, the best formulation contained Medium199, glutathione and maltodextrin. For the trivalent vaccine it is therefore probably necessary to spray dry the different serotypes separately and mix the dry powders afterwards to obtain the trivalent vaccine.

## 1. Introduction

Poliomyelitis is a highly infectious disease, caused by at least one of the three serotypes of poliovirus, the disease can be prevented through vaccination. Live attenuated oral polio vaccine (OPV, based on Sabin strains) is widely used because of its effectiveness, low cost and ease of administration. However, a major concern with the use of OPV is generation of vaccine-derived poliovirus by reversion of the attenuated OPV, causing rare vaccine associated paralytic poliomyelitis (VAPP) [1–3]. Inactivated polio vaccine (IPV, based on wild type Salk strains) is safe, but much more expensive because the purification requires more unit operations (e.g. chromatography) and the vaccine dose needs to be higher due to the inability of the inactivated vaccine particles to replicate after administration.

To achieve global eradication of polio (both wild-type polio viruses as well as vaccine-derived viruses), the Global Polio Eradication Initiative (GPEI) has defined an endgame strategy. This includes a phased withdrawal of OPV and global inclusion of IPV into all routine

vaccination programs [4]. Besides the changes in the existing immunization programs, more affordable, efficacious and safely manufactured polio vaccines are required. The GPEI is pursuing initiatives to minimize IPV costs for developing countries by introducing low-cost IPV based on Sabin strains, instead of Salk strains [5,6].

A drawback of both OPV and IPV, which are marketed as liquid vaccines, is the requirement of a cold chain for their transport and storage. Maintenance of the cold chain is challenging, especially in developing countries, where these vaccines are needed the most [7]. For use in emergency vaccination and post-eradication stockpiling, a thermally stable formulation is strongly desired to maintain vaccine potency during storage and transport.

A potential strategy to stabilize vaccines is to dry them in the presence of stabilizing excipients. Removal of water and incorporation of excipients can improve the stability of vaccines due to decreased mobility and prevention of degradation pathways that are facilitated by water [8,9]. A previous study by Kraan et al. [10] showed that freeze-drying is suitable to stabilize traditional Salk IPV, producing a

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thermostable vaccine in form of dried cakes. Spray drying may be an attractive alternative for freeze-drying because spray dried powder may provide opportunities for new vaccine delivery routes (especially pulmonary and mucosal routes) [11–15]. However, careful selection of the formulation matrix (sugars, polymers, amino acids and surfactants) is required to spray dry labile vaccines in order to retain their potency during and after spray drying [16]. Potency correlates with the presence of D-antigen. Only D-antigen induces poliovirus neutralizing antibodies. Under stress conditions the particle loses D-antigen epitopes and, as a result, its ability to induce functional antibodies.

In this study, we describe the search for a formulation of a dry sIPV produced by spray drying. This was done by (i) studying the influence of process stress elements on sIPV, (ii) an excipient screening aiming to minimize the loss of sIPV D-antigenicity upon drying (and subsequent reconstitution), and (iii) investigating the effects of various excipients on the thermostability of dry sIPV. Furthermore, a fractional factorial design was developed around the most promising formulations to elucidate the contribution of each excipient in stabilizing D-antigen.

## 2. Materials and methods

### 2.1. Vaccine

Monovalent Sabin IPV bulk material used in this study was produced as described previously [17]. The monovalent IPV bulk concentration was 476, 382 and 323 D-antigen units (DU) per mL for type 1, type 2 and type 3, respectively. D-antigen is the native conformational state of the polio virus particle. Only virus and vaccine with D-antigenicity is able to induce virus neutralizing antibodies.

### 2.2. Excipients

The excipients D-trehalose dihydrate, sodium citrate dihydrate, L-arginine, dextrin (maltodextrin) from maize starch (10) were purchased from Sigma (USA). Magnesium chloride hexahydrate and L-glutamic monosodium salt monohydrate (MSG) were from Merck (Germany), Medium199 from Biltoven Biologicals (The Netherlands), Pluronic® F-68 and PBS (0.01 M, pH 7.2) from Gibco life technology (USA).

### 2.3. Dialysis

Unless otherwise indicated, the monovalent IPV bulk material was dialyzed against PBS (0.01 M, pH 7.2) using a 10 kDa molecular weight cut-off, low binding regenerated cellulose membrane dialysis cassette (Slide-A-Lyzer®, Pierce, Thermo Scientific, USA) to replace the buffer components of the sIPV bulk (M199 medium). PBS has earlier [18,19] been shown to be a suitable buffer for spray drying of inactivated viral vaccines.

### 2.4. Formulation preparation

For spray drying trivalent sIPV, all excipients were dissolved in PBS and pH was adjusted to 6.8. In case of spray drying with monovalent sIPV, excipients were also dissolved in PBS and pH was adjusted to 6.8 for type 1 and 2 and pH 6.4 for type 3. The dialyzed sIPV (monovalent or trivalent) was added to the liquid formulation to a final concentration of 10, 16 and 32 DU for type 1, type 2, and type 3 respectively before drying. PH settings were based on prior studies (data not shown).

### 2.5. Spray drying process

Sabin IPV powders were produced using a Büchi mini spray-drier B-290 in conjunction with a high performance cyclone and a B-296 dehumidifier (both from Büchi Labortechnik AG). All the experiments were performed in a closed loop configuration using nitrogen as drying

medium. A two-way nozzle with orifice diameter of 0.7 mm was used in a co-current mode. The nitrogen pressure was set constant at 5 bar. The spray drying parameters were fixed after initial optimization. These fixed settings were as follows: inlet air temperature 110 °C; feed flow rate 3.1 mL/min; atomizing airflow 12.4 L/min (corresponding to an equipment setting of 40 mm); and an aspirator rate of 22 m<sup>3</sup> n/h; this corresponded to an instrument setting of 100%.

After spray drying the spray dried product was collected and aliquoted (100 mg) in vials (3 mL vial, Nuova Ompi) in a glove box (Terra Universal Inc, Series 100) under a relative humidity < 3% as measured by relative humidity analyzer [20]. The vials were subsequently sealed.

### 2.6. D-antigen ELISA

The D-antigen ELISA was performed as described by Westdijk et al. [21]. Polystyrene 96-well plates (Greiner, Austria) were coated overnight at room temperature with bovine anti-polio serum (Biltoven Biologicals, The Netherlands) and blocked with 1% BSA (Sigma–Aldrich) for 30 min at 37 °C. The plates were washed 3 times with PBS (0.01 M) containing 0.05% Tween 80 (Merck, Germany). A series of eight twofold dilutions of IPV-formulation (in triplicate) in 0.01 M PBS containing 0.05% Tween 80 was added to each plate and incubated at 37 °C for 2 h. The unbound antigen was then removed and the plates were washed as above. Type-specific monoclonal antibodies [mAb Hyb295-17-02 (type 1, Thermo scientific), Hyb294-06-02 (type 2, Thermo scientific) and 4-8-7 (type 3, Biltoven Biologicals)] were added and the plates were incubated at 37 °C for 2 h. For trivalent sIPV excipient screening, Hyb300-05-02 (type3, Thermo scientific) was used. After washing the plate, HRPO-conjugated goat anti-mouse IgG (Southern Biotech) was added to each well, followed by incubation at 37 °C for 2 h. Plates were then washed and tetramethylbenzidine (TMB) substrate (Sigma–Aldrich) was added. After 10 min the reaction was stopped by addition of 0.2 M H<sub>2</sub>SO<sub>4</sub> and absorbance at 450 nm was measured with Synergy Mx plate reader (BioTek, USA). Assay data were analyzed by four-parameter logistic curve fitting with Gen5 software. D-antigen units were calculated relative to the reference standard. Unless stated otherwise, D-antigen recovery values were calculated by using the liquid formulations prior to spray drying as the 100%-reference.

### 2.7. Residual moisture content (RMC)

The RMC of spray dried sIPV vaccine samples was determined using a C30 Compact Karl Fischer Coulometer (Mettler-Toledo). Samples of approximately 100 mg dried powder vaccine were reconstituted in 1 mL HYDRANAL Coulomat A (Sigma-Aldrich) and subsequently 100 µL was injected into the titration vessel. Each sample was measured in triplicate. The relative moisture content was calculated using a reference curve of H<sub>2</sub>O in Coulomat A, the weight of the dried product in the vial, the ratio between the volume for reconstitution (1 mL) and injection into the titration vessel (100 µL).

### 2.8. Geometric particle size

The geometric particle size (X<sub>50</sub> defined as the median particle size) of the spray dried powder products was analyzed on the following day (24 h) after spray drying by laser diffraction with a Helos system (Sympatec GmbH). The powder was dispersed into the Helos system using an aspirator dispersing system operated at a dispersing pressure of 1.0 bar. The vaccine powder was measured with a lens having a measuring range of 0.1/0.18–35 mm. Furthermore, to check for any aggregates the analysis was repeated by increasing the dispersing pressure. Increasing the dispersion pressure up to 5.0 bar did not result in a change of the measured particle size distribution, which indicated that the size distribution of the primary particles was obtained upon performing the analysis at 1.0 bar. Results are the mean of three

independent measurements.

### 2.9. Design of experiments (DoE)

The DoE model was prepared and evaluated using MODDE 12.0 software (Umetrics, Sartorius). Models were fitted with multiple linear regression (MLR) and adjusted by removing non-significant model terms. The screening experiment for trivalent sIPV used a full factorial design, consisting of 19 experimental runs. For studies requiring investigation of relative contribution of excipients to monovalent serotypes, a fractional factorial design was used, consisting of in total 11 experimental runs. The choice of a fractional factorial design instead of a full factorial design (requires 19 runs) was made because of efficiency as fewer experimental runs would give the same amount of information and will require less sIPV. To reduce systematic errors, all the experiments were randomized.

## 3. Results and discussion

### 3.1. Impact of shear stress during spray drying on trivalent sIPV activity

The impact of atomization shear stress, on the D-antigenicity of the trivalent IPV was investigated. Shear stress may occur when the vaccine and excipient containing liquid is atomized into small droplets, which may reduce antigen activity. To understand the impact of shear stress in our settings, the nozzle was taken out of the drying system and trivalent sIPV containing 10/16/32 DU of serotype 1, 2 and 3 respectively, was atomized at 12.4 L/min and a pump speed of 3.1 mL/min. This setup excluded any effect of heating as for this experiment the nozzle was placed outside the heating chamber. The generated aerosols were collected and analyzed for the loss in DU compared to the starting bulk. The D-antigen recovery was  $\geq 90\%$  for all serotypes and the confidence intervals also included a full D-antigen recovery (see Fig. 1) indicating only a minor D-ag loss ( $< 10\%$ ) due to atomization. In a previous study on viral vectors based on adenovirus [22], an increase in atomization pressure (7.3 L/min to 11.2 L/min) increased the loss in viral infectivity. In this study, however, no negative effect of the atomization on the D-antigen recovery of the viral vaccine was detected. Consequently, an atomization rate of 12.4 L/min and pump speed of 3.1 mL/min were selected for further experiments.

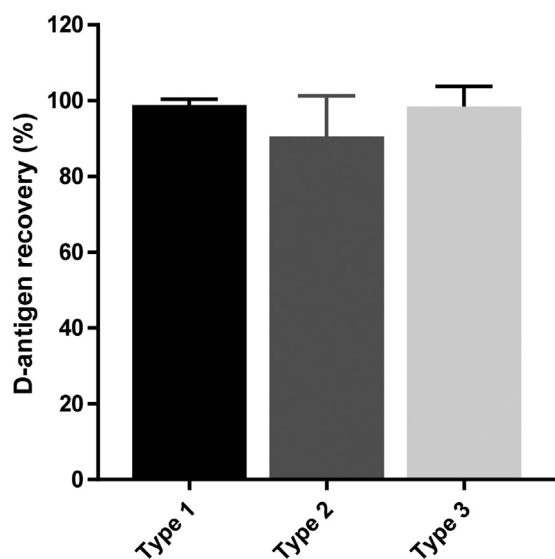


Fig. 1. Effect of atomization stress on the D-antigen recovery (%) during spray drying of trivalent sIPV vaccine. The investigated setting for nozzle shear stress was 12.4 L/min and the feed flow rate was 3.1 mL/min. Average values  $\pm \sigma_{n-1}$  ( $n = 3$ ) are depicted.

### 3.2. Impact of dehydration stress during spray drying on trivalent sIPV

To investigate the influence of dehydration stress on sIPV D-antigen, a base formulation was selected, consisting of two components, the non-reducing disaccharide trehalose (10% w/v) and magnesium chloride hexahydrate (3% w/v). The pH of the formulation was adjusted to 6.8. The trivalent sIPV vaccine (10/16/32 DU for type 1, 2 and 3 respectively) in the base formulation was spray dried at three different drying temperatures and feed flow rates (Fig. 2). The D-antigen recovery of sIPV was affected only to a limited extent within the three tested process conditions. However, the differences between the recovery of the various serotypes was substantial (Fig. 2). In contrast to atomization, dehydration resulted in substantial D-antigen decrease of sIPV. As a result, dehydration was identified as the main cause for a loss in D-antigenicity during spray drying. Serotype 1 was least affected by drying, followed by serotype 3 and 2. Considering the above observations and the objective to attain maximum D-antigen recovery and low residual moisture content, an atomization rate of 12.4 L/min, pump speed of 3.1 mL/min and an inlet drying temperature of 110 °C was selected for all further studies. It is important to keep the outlet temperature of the process below 55 °C because above that temperature the native D-antigen conformation of the vaccine changes to the non-native C-antigenic conformation. Thus, to keep this risk at minimum we choose the temperature conditions (inlet temperature 110 °C) and thus outlet temperatures varying between (48–53 °C).

### 3.3. Excipient screening for spray drying trivalent sIPV

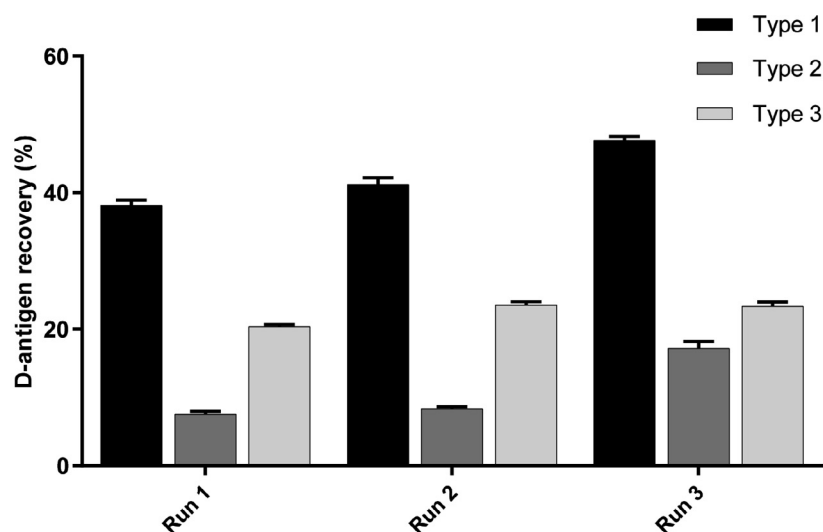
To obtain a sIPV formulation with an improved antigenic recovery during spray drying, excipient screening using a DoE approach was performed. The preceding base formulation was selected along with monosodium glutamate and mannitol based on findings from the literature [10]. A full factorial design (Table 1) was performed around the excipients. In general, the addition of monosodium glutamate (EXP 8, Table 1) and mannitol (EXP 12, Table 1) did not further improve the D-antigen recovery during drying compared to the base formulation (EXP 6, Table 1). Serotype 1 in the formulations was least affected during spray drying, with the best recovery of 46%, followed by 17% for serotype 2 and 22% for serotype 3.

The residual moisture content varied between 2 and 13%. The powder particle size varied between 3.6 and 6.1  $\mu\text{m}$ , with formulation containing trivalent vaccine and seemed to be an effect of changing excipient concentration. Increasing the dispersion pressure from 1 to 5 bars during particle size analysis, did not result in decreased powder particle size. Thus, it can be assumed that the vaccine powder was not aggregated during the initial storage. When designing the powder for a particular route of administration (for example alveolar deposition via the pulmonary route requires particle size b/w 1–5  $\mu\text{m}$ ), additional optimization may be required.

Overall, D-antigen recoveries were low for all serotypes. Screening of different excipient combinations during sIPV spray drying indicated a dependency between formulation composition and process loss. Moreover, the D-antigen recovery data indicates that each serotype was affected differently by the same formulation. For that reason, it was decided to formulate each individual serotype. Thus, further experiments were carried out with monovalent serotypes which were dried using the same fixed spray drying conditions.

### 3.4. Stabilizing monovalent Sabin IPV serotypes

Stabilizing formulations were different mixtures, containing one or more of the following components: (i) a non-reducing disaccharide (trehalose), (ii) a polysaccharide (maltodextrin), (iii) divalent metal ion ( $\text{Mg}^{2+}$ ), (iv) amino acids (monosodium glutamate, L-arginine, L-glutathione and glycine), (v) sodium salt of an organic acid (citrate), (vi) cell culture medium (M199) and (vii) a surfactant (Pluronic®). The



Inlet air temperature (°C)	80	90	135
Feed flow rate (mL/min)	1.0	2.5	1.5
Outlet temperature (°C)	50	51	77
RMC (%)	11.6 ± 0.2	13.5 ± 0.5	6.2 ± 0.1

Fig. 2. Effect of spray drying conditions on the D-antigen recovery of trivalent sIPV vaccine. The atomization rate was kept constant for all runs (12.4 L/min). Average values  $\pm \sigma_{n-1}$  (n = 3) are depicted.

spray drying solution consisted of a concentration of 10/16/32 D-antigen units for monovalent serotype 1/2/3, respectively. D-antigen recovery after drying and after 1 week storage at 40 °C were determined.

In general, spray drying transformed all the liquid formulations (described in Table 2) into fine powders with a particle size of 5–7  $\mu\text{m}$  (Suppl. Table 1). The D-antigen recovery data in Table 3 indicate an acceptable process related loss in D-antigenicity of 2% for serotype 1 and 21% for serotype 2. However, the loss after spray drying was considerably higher (44%) for sIPV serotype 3. The formulation composition affected both the residual moisture content in the spray dried powders and the process loss. The RMC of the powders varied between 1 and 7% (Table 3). However, there was no apparent correlation between either process loss or storage stability and RMC in the observed

range of RMC. The high RMC for dried sIPV formulations could partially be explained by the presence of  $\text{MgCl}_2$ , which is likely present in the crystalline hexahydrate form. Omitting  $\text{MgCl}_2$  from the formulation and fine tuning of the drying process, could decrease the RMC to a level recommended for dried biologicals by European Pharmacopoeia (< 3%).

Each monovalent serotype required a specific formulation composition amenable for spray drying to reach its maximum D-antigenicity. For serotype 1 both trehalose 20% (w/v) and maltodextrin 20% (w/v) performed as good bulking agents, attaining a maximum D-antigen recovery of 97% and 98% respectively (EXP S1 and S4, Table 3). While for stabilizing sIPV serotype 2, maltodextrin in combination with L-arginine 4% (w/v) appeared to be favorable as indicated by the D-

Table 1

Excipient screening for spray drying of trivalent sIPV. The effect of spray drying various formulations on the D-antigen recovery of sIPV type 1, 2, and 3 is shown. Also included are the residual moisture content (RMC, %) and geometric particle size ( $X_{50}$ ,  $\mu\text{m}$ ) of the spray dried, average values (n = 3) are given. The symbol \*): Insufficient powder (powder yield < 10%) for analysis, due to sticking in the drying chamber.

EXP	Component (w/v, %)				D-antigen recovery (%)			RMC (%)	Particle size $X_{50}$ ( $\mu\text{m}$ )
	Trehalose	MSG	$\text{MgCl}_2$	Mannitol	Serotype 1	Serotype 2	Serotype 3		
1	5	0	0	0	22	5	16	2.7 ± 0.0	3.7 ± 0.1
2	10	0	0	0	25	4	16	3.2 ± 0.1	4.0 ± 0.1
3	5	3	0	0	18	5	16	2.9 ± 0.1	3.6 ± 0.2
4	10	3	0	0	20	10	17	2.2 ± 0.2	4.1 ± 0.2
5	5	0	3	0	38	6	20	13.0 ± 0.1	3.9 ± 0.1
6	10	0	3	0	46	17	22	6.2 ± 0.1	3.9 ± 0.1
7	5	3	3	0	45	11	20	2.7 ± 0.2	5.0 ± 0.3
8	10	3	3	0	44	12	22	4.6 ± 0.1	4.9 ± 0.5
9	5	0	0	5	–*	–	–	–	–
10	10	0	0	5	–	–	–	–	–
11	5	3	0	5	–	–	–	–	–
12	10	3	0	5	40	5	12	1.5 ± 0.3	6.1 ± 0.1
13	5	0	3	5	25	6	14	4.3 ± 0.3	4.9 ± 0.1
14	10	0	3	5	4	0	0	3.8 ± 0.2	5.1 ± 0.3
15	5	3	3	5	18	0	12	3.3 ± 0.3	4.9 ± 0.4
16	10	3	3	5	19	0	10	2.6 ± 0.1	5.1 ± 0.2
17	7.5	1.5	1.5	2.5	21	5	8	2.5 ± 0.1	4.0 ± 0.0
18	7.5	1.5	1.5	2.5	18	2	7	2.8 ± 0.1	4.0 ± 0.1
19	7.5	1.5	1.5	2.5	20	2	4	2.2 ± 0.1	4.1 ± 0.1

**Table 2**

Composition of evaluated formulations for spray drying. Formulations containing a monovalent sIPV type 1 (S1-S6), 2 (S1-S6), or 3 (S7-S9). A-G (arbitrary letters to segregate component categories).

EXP	Formulation composition						
	A (w/v)	B (w/v)	C (w/v)	D (w/v)	E (w/v)	F (v/v)	G (w/v)
S1	20% trehalose	–	8% MgCl <sub>2</sub>	8% monosodium glutamate	–	–	3.87% monosodium citrate
S2	10% trehalose	–	8% MgCl <sub>2</sub>	4% L-arginine	–	–	3.87% monosodium citrate
S3	10% trehalose	–	8% MgCl <sub>2</sub>	4% L-arginine	0.03% Pluronic	–	3.87% monosodium citrate
S4	–	20% maltodextrin	8% MgCl <sub>2</sub>	8% monosodium glutamate	0.03% Pluronic	–	–
S5	–	20% maltodextrin	8% MgCl <sub>2</sub>	4% L-arginine	–	–	3.87% monosodium citrate
S6	–	20% maltodextrin	8% MgCl <sub>2</sub>	4% L-arginine	0.03% Pluronic	–	3.87% monosodium citrate
S7	–	20% maltodextrin	–	8% monosodium glutamate 0.62% L-glutathione 4.5% glycine	–	90% M199	–
S8	–	20% maltodextrin	–	0.62% L-glutathione 4.5% glycine	–	90% M199	3.87% monosodium citrate
S9	–	20% maltodextrin	8% MgCl <sub>2</sub>	8% monosodium glutamate 0.62% L-glutathione 4.5% glycine	–	90% M199	3.87% monosodium citrate

antigen recovery of 79% (EXP S6, Table 3). Trehalose and maltodextrin probably exert their protective mechanism by immobilization of vaccine in an amorphous matrix portrayed by the vitrification theory [23]. Trehalose has been proven to stabilize both attenuated and inactivated viral vaccines during spray drying including measles [24], influenza [18,25] and human papilloma virus [26]. While, maltodextrin has also been previously used to stabilize Salk IPV in a formulation used for vacuum drying recovering roughly half of the starting D-antigen concentration [27]. However, it is important to note that Salk strains of IPV are different from Sabin IPV in respect to isoelectric points [28] and capsid structure (amino acid sequence) that may affect its stability during drying.

Inclusion of the surfactant Pluronic F68, did not contribute to the D-antigen recovery of serotype 2, however, it did improve the D-antigen recovery of serotype 1 by 24% (EXP S5 vs EXP S6, Table 3). Serotype 3 required a formulation containing cell culture medium (M199), which is a complex medium with approximately 60 components in various concentrations including amino acids (0.004–0.002% w/v), vitamins (0.00001–0.000001% w/v) and inorganic salts (0.8–0.0007% w/v). Table 3 shows type 3 D-antigen recoveries between 40 and 60% (EXP S7-S9).

The thermal instability of liquid sIPV was clearly evident as the D-antigenicity of all three unformulated monovalent liquid serotypes in PBS was undetectable, when stored at 40 °C for 1 week (Liquid, Table 3). However, serotype 1 formulated in the lead formulations S1 and S4 showed no loss in antigenicity during incubation at 40 °C, maintaining 97% and 98% DU recovery. A decrease of 18% in

antigenicity was observed for serotype 2 lead formulation S5. It was interesting to note that the antigenicity of serotype 2 in the S3 formulation had decreased to 52% during drying, but decreased further by only 13% during storage. This was better than observed for the lead formulation S5. The strongest decrease in antigenicity after storage was observed for serotype 3, where a decrease of 37% in DU from its initial value was observed for lead formulation S8.

### 3.5. Elucidating the relative contribution of stabilizing excipients

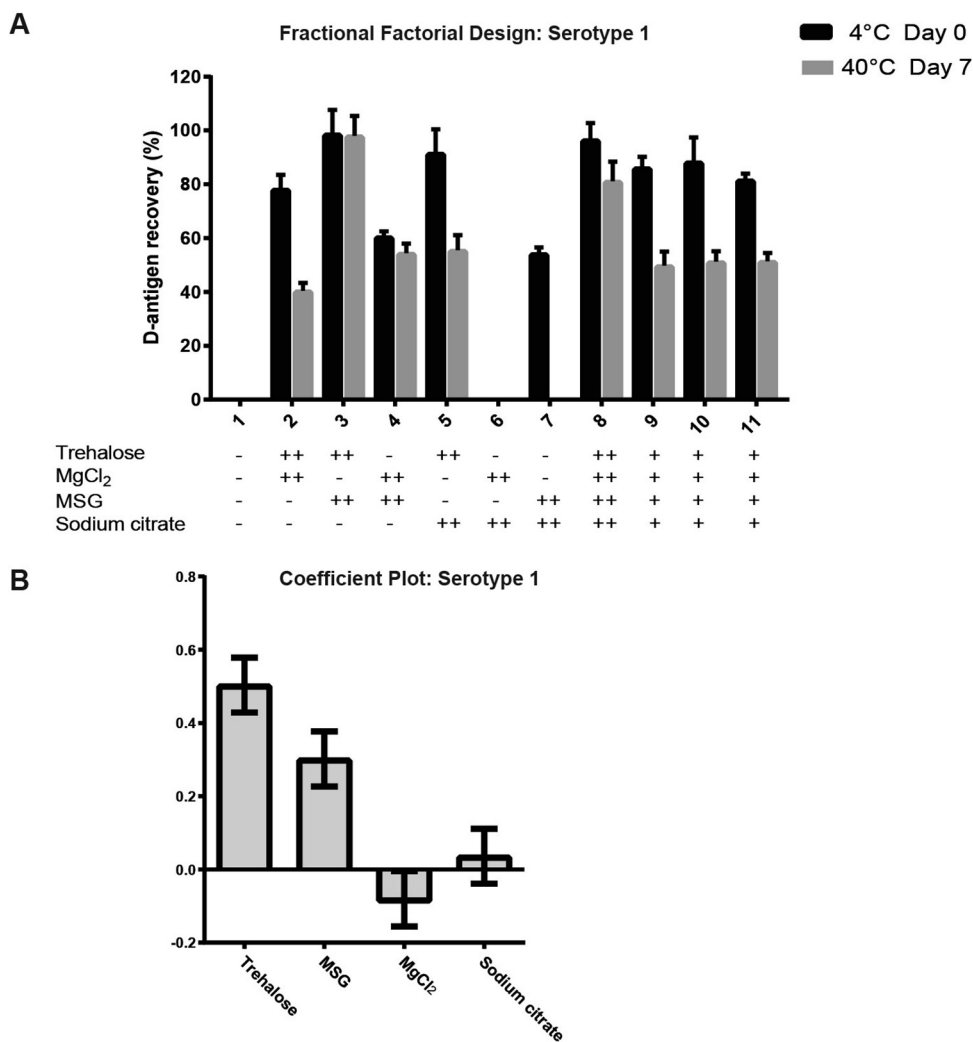
Based on the results of our study so far, the most promising formulations were selected for further investigation to get more insight into the impact of excipients on the stabilization of sIPV using a DoE approach. Moreover, according to ICH Q8 guidelines for pharmaceutical development, only the excipients whose use could be justified should be included in a formulation [29]. Thus to narrow down the excipients that contribute to the D-antigen recovery and/or stability, a design of experiment approach was used. Therefore, a fractional factorial design was developed around the lead formulations S1 (for serotype 1, Fig. 3A), S5 (for serotype 2, Fig. 4A) and S8 (serotype 3, Fig. 5A). The formulations were spray dried and subsequently stored for 1 week at 40 °C and analyzed to assess the changes in D-antigen recovery during storage. The recovery (both after drying and storage) was calculated in reference to the liquid vaccine control.

Multiple linear regression (MLR) models were fitted to the D-antigen recovery data for sIPV serotype 1, 2 and 3, which resulted in valid models (serotype 1:  $R^2 = 0.89$ ,  $Q^2 = 0.95$ ; serotype 2:  $R^2 = 0.77$ ,

**Table 3**

Effect of spray drying various formulations (see Table 2 for composition) on the D-antigen recovery of sIPV type 1, 2, and 3. Also included is the residual moisture content (RMC, %) of the dried powder. The thermal stability was studied by determining the D-antigen recovery after storing the dried powder for 1 week at 40 °C (calculated in comparison to the starting material before drying). The symbol “–” depicts experiment not performed.

EXP	After spray drying (t = 0)						After 1 week storage at 40 °C		
	Type 1 (%)		Type 2 (%)		Type 3 (%)		Type 1 (%)	Type 2 (%)	Type 3 (%)
	Recovery	RMC	Recovery	RMC	Recovery	RMC	Recovery	Recovery	Recovery
Liquid	–	–	–	–	–	–	0	0	0
S1	97	4.2 ± 0.0	46	5.5 ± 0.0	–	–	97	32	–
S2	82	7.8 ± 0.4	55	5.4 ± 0.1	–	–	59	28	–
S3	68	7.9 ± 0.4	52	5.1 ± 0.2	–	–	68	39	–
S4	98	3.3 ± 0.1	71	4.5 ± 0.4	–	–	98	52	–
S5	53	6.6 ± 0.2	78	5.6 ± 0.2	–	–	24	60	–
S6	77	4.8 ± 0.1	79	6.0 ± 0.4	–	–	45	54	–
S7	–	–	–	–	56	2.8 ± 0.1	–	–	5
S8	–	–	–	–	56	1.6 ± 0.1	–	–	19
S9	–	–	–	–	42	2.1 ± 0.1	–	–	0



**Fig. 3.** (A) Fractional factorial design used for sIPV type 1. Trehalose (tested range 0–20% w/v), MSG (tested range 0–8% w/v), Sodium citrate (tested range 0–3.87% w/v) and MgCl<sub>2</sub> (tested range 0–8% w/v). Signs (–) represents no excipient, (+) represents middle concentration and (++) represents maximum concentration of investigated range. (B) The main excipient effects that contribute to the best fitted model (for sIPV serotype 1) are shown in coefficient plot (D-antigen recovery after spray drying).

$Q^2 = 0.73$  and serotype 3:  $R^2 = 0.94$ ,  $Q^2 = 0.69$ ) to predict D-antigen recoveries directly after spray drying.  $R^2$  indicates the model fit (Goodness of fit, 1 = perfect model) and  $Q^2$  the prediction power of the model (Goodness of prediction, values greater than 0.5 is a good fit). The effect of different excipients (excluding non-significant parameters) on D-antigen recovery for sIPV type 1 2 and 3 after spray drying are depicted in Figs. 3B, 4B and 5B, respectively. For type 3, the M199 to maltodextrin ratio was kept constant in the design and other excipients were varied.

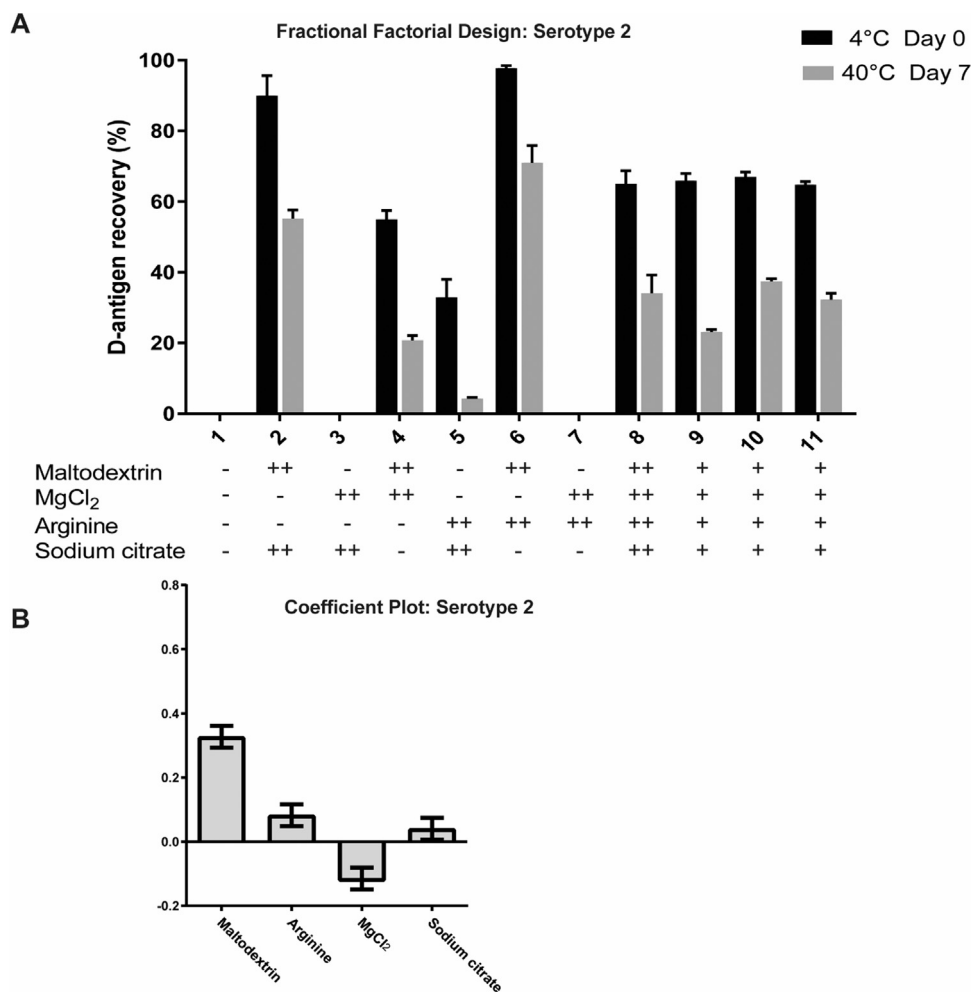
For serotype 1 trehalose and monosodium glutamate positively affect the D-antigen recovery (see Fig. 3B). In the absence of any excipient, almost no powder could be recovered after spray drying (EXP 1, Fig. 3A). Both trehalose and monosodium glutamate were able to retain 98% D-antigen for serotype 1 that was further maintained during storage at elevated temperatures for 1 week (EXP 3, Fig. 3A). Interestingly, absence of either of these components affects the D-antigen recovery and absence of both trehalose and monosodium glutamate leads to a complete loss of D-antigenicity (EXP 6, Fig. 3A). An explanation for this may be that excluding the matrix solute in the feed affects the ability to encapsulate the bioactive agent which is required to retain bioactivity during the drying process and subsequent storage. The coefficient plot (Fig. 3B) obtained from the model indicate that omitting MgCl<sub>2</sub> from the formulation and optimizing the concentration of trehalose and MSG

could lead to a formulation with a minimum number of excipients and maximum D-antigen recovery.

A similar stabilizing trend was observed for serotype 2. Maltodextrin and arginine were important to prevent D-antigen deterioration during spray drying. The combination of maltodextrin and arginine lead to the highest D-antigen recovery of 97% after drying for serotype 2 (experiment 6, Fig. 4A). However, on storage a decrease of 26% in antigenicity was detected. Absence of both maltodextrin and arginine (EXP 3, Fig. 4A) leads to complete loss of antigenicity. The coefficient plots obtained from the model for serotype 2, indicate that omitting MgCl<sub>2</sub> and optimizing the maltodextrin and arginine concentration for obtaining a formulation with minimum number of excipients and a maximum D-antigen recovery.

The stabilizing potential of L-glutathione for serotype 3, during spray drying was evident from the coefficient plots (Fig. 5B). Moreover, the coefficient plots indicated that MSG positively influenced the D-antigen recovery. The plots obtained from the model further indicated toward excluding MgCl<sub>2</sub> and optimizing the L-glutathione and MSG concentration to achieve the optimal D-antigen recovery. Considering that the maximum recoveries of serotype 3 obtained were moderate when compared to the other serotypes, the formulation requires further optimization.

Amino acid like monosodium glutamate (8% w/v) or arginine (4%



**Fig. 4.** (A) Fractional factorial design used for sIPV type 2. Maltodextrin (tested range 0–20% w/v), Arginine (tested range 0–4% w/v), Sodium citrate (0–3.87% w/v) and MgCl<sub>2</sub> (0–8% w/v). Signs (–) represents no excipient, (+) represents middle concentration and (++) represents maximum concentration of investigated range. (B) The main excipient effects that contribute to the best fitted model (for sIPV serotype 2) are shown in coefficient plot (D-antigen recovery after spray drying).

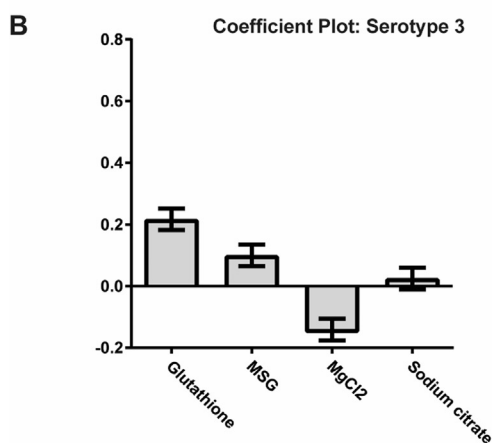
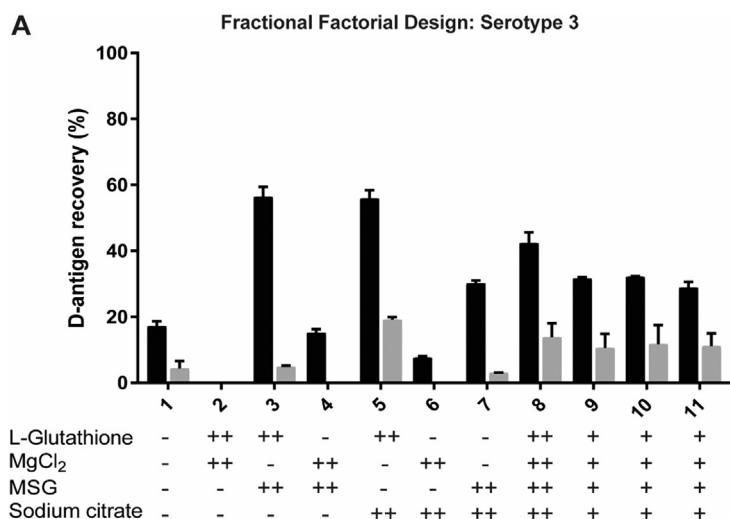
w/v) positively contributed to the D-antigen recovery of all three serotypes (Figs. 3B, 4B and 5B). Use of arginine has been reported to be beneficial for spray drying of live attenuated measles virus vaccine [24]. Moreover, it has been shown to reduce protein–protein interactions, thereby reducing aggregation [30]. This could be the mechanism through which sIPV is stabilized during spray drying. In addition, L-glutathione (0.62% w/v) positively contributed to D-antigenic recovery of serotype 3 during spray drying. The stabilizing effect may be due to the direct interaction of L-glutathione with the viral capsid [31].

In previous studies, divalent cations like Mg<sup>2+</sup> have shown to be beneficial for liquid live attenuated oral polio vaccine [32]. Chen et al. [33] described that MgCl<sub>2</sub> stabilizes poliovirus conformation by specific ion interaction with capsid proteins. Another study from Kraan et al. [10] investigating freeze-drying of Salk IPV in vials and bioneedles [34], showed that inclusion of MgCl<sub>2</sub> improved the D-antigen recovery of all three serotypes, especially upon storage after drying. However, the current study shows that MgCl<sub>2</sub> was not beneficial for spray drying of sIPV. From the three fractional factorial designs investigating the serotypes 1, 2 and 3 respectively, it may be concluded that MgCl<sub>2</sub> was an unfavorable excipient candidate for stabilization of sIPV during spray drying and storage. This apparently contrasts with the favorable effect of Mg<sup>2+</sup> on the thermostability of Sabin poliovirus in the liquid state [33]. Thus, to minimize the complexity of the formulation, magnesium chloride should be excluded from the formulation. In addition, it was found that every serotype could be reproducibly spray dried with similar D-antigenic recoveries (EXP 9, 10 and 11, Figs. 3A, 4A and 5A)

indicating robustness of the drying method.

Despite the large number of excipient combinations evaluated in these studies, it is quite possible that the specific formulations tested here did not include the fully optimized composition. Although it was possible to produce a formulation that had close to 100% recovery after drying for serotype 1 and 2. For serotype 3, this was more challenging as here a maximum D-antigen recovery of 56% was found after spray drying. This might be improved in the future by adding (an) other excipient(s) to EXP 5, Fig. 5A.

The stability during storage at 40 °C for a week varied for each serotype, the observed thermostability decreases in the following order: serotype 1 > serotype 2 > serotype 3. The formulation for serotype 1 [trehalose (20% w/v) and MSG (8% w/v)] could fully retain the D-antigenicity on storage at 40 °C for 1 week. Serotype 2 containing formulation [maltodextrin (20% w/v) and arginine (8% w/v)] showed a slight (18%) decrease in D-antigenicity on storage. Serotype 3 exhibited the most prominent loss in D-antigenicity on storage. It is speculated that the observed thermal instability of sIPV serotype 3 (formalin inactivated virus) is an intrinsic characteristic of the type 3 particle, as this instability (compared to the other two polio serotypes) has also been observed in studies with live attenuated type 3 Sabin virus [35]. The stability was assessed for a week at 40 °C, aiming for a controlled temperature chain approach defined by WHO for use of vaccine outside the cold chain [36]. For an uncontrolled temperature chain extended stability studies should be performed.



**Fig. 5.** (A) Fractional factorial design used for sIPV type 3. L-glutathione (tested range 0–6.2 mg/mL), Monosodium glutamate (tested range 0–8% w/v), Sodium citrate (tested range 0–3.87% w/v) and MgCl<sub>2</sub> (tested range 0–8% w/v). The two excipients that were fixed and included in all the formulations were maltodextrin 20% (w/v) and M199-glycine 90% (v/v). Signs (–) represents no excipient, (+) represents middle concentration (++) represents maximum concentration of investigated range. (B) The main excipient effects that contribute to the best fitted model (for sIPV serotype 3) are shown in coefficient plot (D-antigen recovery after spray drying).

#### 4. Conclusions

This study shows the feasibility and limitations of spray drying sIPV in a tailored formulation for each respective serotype either 1, 2, or 3. Although further improvement is still needed for type 3, these findings show the possibility to produce a spray dried vaccine powder based on safer (with respect to production of vaccine virus) sIPV [37], which could be used for stockpiling and distribution in developing countries without the need of a cold chain transport. In addition, the dried powder formulation provides opportunities for vaccine delivery via alternative routes like the intranasal or sublingual route [38].

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ejpb.2018.05.021>.

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