Novel formulations and delivery strategies for inactivated polio vaccines : new routes with benefits

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Polymer-based oral dissolving films for polio vaccination

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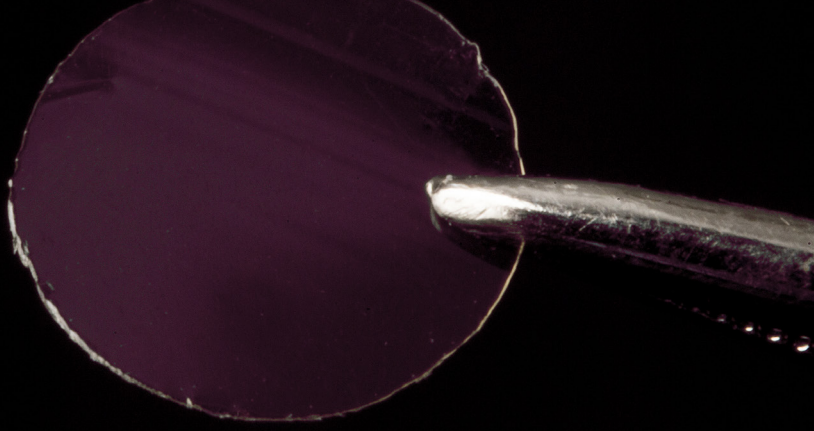

ABSTRACT

Alternative delivery technologies for polio vaccination might be of importance in the development of improved polio vaccines that are needed towards complete polio eradication and their use in the period thereafter. Vaccination via oral mucosa, like the sublingual or buccal route, could be an easy applicable and safe polio immunization approach for routine immunization or as a tool for outbreak intervention after cessation of the live oral polio vaccine.

Aim of the current study was to evaluate the possibility to make polymer-based films containing trivalent sIPV and suitable for oromucosal vaccination. A combination of a Design of Experiments approach and the evaluation of excipients with already proven stabilizing capacity of polio antigens was used to develop sIPV-containing oral film formulations while preserving its D-antigenicity for 85-100% for type 1, 60-85% for type 2, and 50-75% for type 3. This study revealed that a combination of excipients based on sorbitol, magnesium chloride and monosodium glutamate, has strong stabilizing potential for sIPV-films, even when combined with different film formers, i.e., hydroxypropyl cellulose, sodium glutamate or sodium carboxymethyl cellulose. This pivotal study showed the promise of dried polymer-based sIPV-films that might be suitable for sublingual or buccal polio vaccination. Further optimization is required during future product development studies, especially with respect to the mechanical properties of the film formulations.

INTRODUCTION

Since eradication of polio is one of the top global health priorities, the need for inactivated polio vaccines (IPV) that are more affordable, more effective, and safer than existing polio vaccines is higher than ever. This new generation of IPV, for instance based on attenuated Sabin strains, should preferably also induce mucosal immunity, remain stable outside the cold-chain, and be easy to administer. Such a vaccine is valuable with regard to stockpiling and outbreak control in the period after complete cessation of the oral polio vaccine (OPV) and after polio eradication [1]. Vaccination via mucosal sites has the benefits of needle free vaccine delivery and may induce strong mucosal immunity, even at distant effector sites. The induction of polio-specific immunity in the gut protects against polio infection and may interrupt the person-to-person transmission of poliovirus [2].

A previous study in mice revealed that sublingual immunization with fluid Sabin IPV (sIPV) induced systemic poliovirus-neutralizing immune responses as well as polio-specific IgA-producing B cells in the spleen. Moreover, sublingual sIPV delivery elicited polio-specific IgA antibodies at different mucosal sites, where conventional intramuscular vaccination was unable to do so [3]. In the same study, intranasal sIPV vaccination showed to be more efficient in eliciting polio-specific immune responses as compared with the sublingual route [3]. However, besides the concern of uptake by nervous tissue via the olfactory bulbs, which may cause adverse effects (like Bell's palsy), the risk of wheezing in young children exists, making the intranasal route potentially less suitable for infants [1]. Sublingual vaccine delivery has gained significant attention as shown by the numerous studies on this innovative and non-invasive route [4]. For sublingual IPV delivery, the inclusion of an adjuvant may be required to circumvent tolerance or low-to-undetectable immune responses [3, 5]. Besides, novel oral dosage forms that facilitate transport through the oral mucosa, for example by extending the contact time of the antigen, might be desirable for successful vaccination.

Several dosage forms exist for sublingual and buccal delivery of marketed drugs. However, not all of them will be suitable for oral mucosal vaccine delivery and only few have been used to explore sublingual delivery of vaccines. Novel dosage forms that might have the ability to retain the antigen at the sublingual or buccal delivery site are (mucoadhesive) oromucosal films or -tablets, and thermoresponsive gels. Currently, only thermoresponsive gels, which are aqueous solutions at room temperature but transform into gels when at body temperature (e.g., upon contact with the mucosa), have been non- and preclinical evaluated as new dosage form for sublingual delivery of IPV. Sublingual administration of these gels,

containing Salk IPV plus a double mutant heat-labile toxin (dmLT) as adjuvant, induced polio-specific functional antibodies in serum as well as polio-specific IgA antibodies in mucosal samples [5]. Among the solid oral dosage forms are the (mucoadhesive) extended release films and tablets. Converting the vaccine into the dry state might improve the thermostability and could therefore reduce vaccine system costs tremendously. When vaccines no longer require cold storage, or could be kept out of the cold-chain long enough for their transport to remote areas in developing countries, logistic costs will decrease and vaccine availability will be improved [6, 7].

Aim of the current study was to evaluate the possibility to make polymer-based oromucosal films containing trivalent sIPV. First, a target product profile (TPP) describing the desired properties and characteristics of a final product was defined. Among those characteristics are the critical quality attributes (CQAs), which should be within an appropriate limit, range or distribution to ensure the desired product quality, efficacy and safety of the product when used [8, 9]. Further on, different film forming polymers (i.e., hydroxypropyl cellulose (HPC), sodium alginate and sodium carboxymethyl cellulose (CMC)) were selected from literature and tested in combination with excipients that were able to stabilize the antigen during the drying process. D-antigenicity of prepared oral films was assessed in an ELISA directly upon drying. To gain insight into the effects of the main components, i.e. film forming polymer and plasticizers, on both D-antigen recovery and physical/mechanical film characteristics, a Design of Experiments (DoE) approach was used. Subsequently, oral film formulations were further optimized based on D-antigen recovery of each serotype. It was investigated whether the addition of sugars or the combination sorbitol, magnesium chloride and monosodium glutamate could further improve the D-antigen recovery of each serotype in order to yield an oral film formulation with minimal loss of antigenicity during the drying process.

MATERIALS AND METHODS

Materials

Monovalent Sabin IPV bulk material used in this study was produced as described previously [10]. For the preparation of trivalent sIPV, monovalent type 1, type 2 and type 3 were mixed and, subsequently, concentrated using 10 kDa Amicon® Ultra Centrifugal Filters (Merck Millipore, Billerica, MA).

The excipients hydroxypropyl cellulose (HPC), glycerol, D-sorbitol, D-trehalose dehydrate, sucrose, maltodextrin, L-glutamic acid monosodium salt monohydrate (MSG), magnesiumchloride hexahydrate and TRIS (Trizma Base) were purchased from Sigma Aldrich (St. Louis, MO). To prepare 10 mM phosphate buffer, 10 mM disodium hydrogen phosphate heptahydrate (Na2HPO4 * 7H20 from Merck, Darmstadt Germany) was added to 10 mM potassium dihydrogen phosphate (Na2HPO4 from Merck, Darmstadt, Germany) until pH 7.0 was reached. All excipients used were of reagent quality or higher grade.

Methods

Preparation of casting solutions

For the preparation of stock solutions, excipients were dissolved in 10 mM TRIS (for HPC-containing films) or phosphate buffer (SA- or CMC-containing films) (under constant stirring). After complete dissolution, the pH was adjusted to 7.0 ± 0.1 and, subsequently, formulations were mixed with concentrated sIPV bulk using an Intelli-Mixer. Air bubbles were removed by a short spin in an Eppendorf centrifuge. Placebo film formulations were prepared for viscosity measurements and determination of film characteristics as described previously [11]. The viscosity of the casting solutions was measured directly after preparation at ambient temperature using a viscometer (Brookfield, Middleboro, USA). Depending on the viscosity of the casting solution spindle T-B or S04 was used.

Standard film casting method

The solutions were cast onto a release liner (Primeliner® 410/36, Loparex, Apeldoorn, The Netherlands) with a quadruple film applicator using a casting height of 1000 µm. The release liner was fixed to a COATMASTER film casting apparatus (Erichsen, Hemer, Germany) by vacuum suction. The casting speed was 10 mm/s and, subsequently, the film layer was dried at 30°C and at ambient relative humidity. Placebo films were punched using an Artemio perforator (Artemio, Wavre, Belgium) in squares of 1.8 x 1.8 cm, yielding stamp-shaped oral films used in tests for mechanical and physical properties of film formulations as described below.

Ring-based film casting method

Formulations containing sIPV were cast into metal rings (Ø 1.6 cm) in a volume of 201 µL/ring (corresponding with casting height of 1000 µm) onto polyethylene terephthalate foil (Silphan S75 M371, Siliconature, Treviso, Italy) as release liner (see Figure 1B). CMC-containing films were cast in a volume of 301 µL/ring (casting height 1500 µm)), since films with holes were obtained when casting at 1000 µm casting height. Subsequently, the film layer was dried at 30°C and ambient relative humidity for up to 20h. This novel ring-based method was developed to avoid spreading or shrinkage of the liquid formulations with low viscosity and allow film casting using small volumes to minimize the amount of antigen needed.

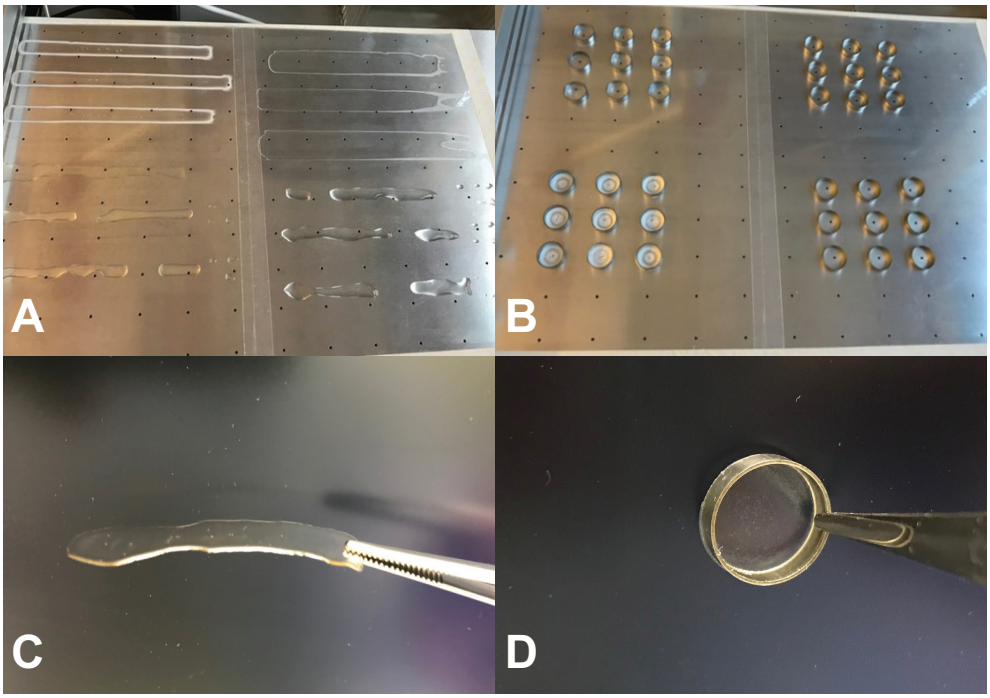

Figure 1 Preparation of films was performed using different film production methods. Since standard film casting method using a film casting apparatus (A) resulted sometimes in spreading (A: upper right formulation) or shrinkage (A: lower formulations) of the liquid formulations with low viscosity, a ring-based film casting method (B) was developed. sIPV-containing oral film formulations consisting of sodium carboxymethyl cellulose, sorbitol, magnesium chloride and monosodium glutamate resulted in transparent films that were easily removable from the release liner, either when cast with the standard (C) or ring-based film casting method (D). Due to shrinkage of the casting solution on the release liner directly upon standard casting, an irregular shaped film is formed (C), whereas the ring-based method yield a more uniform film surface.

Physical and mechanical characterization of oral films

Placebo film formulations were prepared to evaluate physical and mechanical properties of oral films. The thickness was measured using a microscrew meter (Mitutoyo, Neuss, Germany) at five different points of the prepared film. Uniformity of mass was determined according to the Ph. Eur. 9th edition: uniformity of mass for single-dose preparations. Twenty randomly chosen oral films were weighed individually on an analytical balance and average mass was calculated. Residual water content of placebo film formulations was measured using an infrared moisture analyzer (Sartorius MA40, Sartorius Göttingen, Germany). Approximately 1.5 gram of oral films were weighed and heated at 105°C for at least 1.5h until equilibrium in weight was reached. Loss on drying was calculated as the difference (in %) in mass between the initial weight and the final weight at equilibrium:

$$\text{Loss on drying} = \frac{\text{Initial film weight (t=0) - Film weight (t=1.5h)}}{\text{Initial film weight (t=0)}} \times 100 \qquad (1)$$

The mechanical properties of oral films were analyzed as described previously [11]. A minimum of six punched films were tested using an Instron series 5500 load frame with a load cell of 100N (Instron, Norwood, USA). Films were fixed between two clamps that moved away from each other with a crosshead speed of 50 mm/min until film tearing or breakage. Tensile strength (N/mm2), Young's modulus (N/mm2) and elongation at break (%) were calculated using the following equations:

$$\text{Tensile strength} = \frac{\text{load at auto break}}{\text{cross-sectional area of film}} \times 100 \qquad (2)$$

$$\text{Young's modulus} = \frac{\text{slope of stress-strain curve}}{\text{film thickness x cross-head speed}} \qquad (3)$$

$$\text{Elongation at break} = \frac{\text{increase in length at break}}{\text{initial film length}} \times 100 \qquad (4)$$

D-antigen ELISA

Immediately after drying, sIPV-containing films were dissolved and analyzed for antigenically active D-antigen by ELISA as described earlier with some small adaptations [12]. Microtiter plates were coated overnight with serotype-specific bovine anti-polio serum

and, subsequently, blocked with 1% bovine serum albumin (Sigma Aldrich, St. Louis, MO) in PBS for 30 min at 37°C. After washing with 0.05% Tween 80 (Merck, Darmstadt, Germany) in PBS, serial dilutions of sIPV samples (reconstituted films or liquid controls) were added and incubated for 2h at 37°C. Subsequently, plates were washed and serotype-specific monoclonal antibodies (HYB295-17-02 (type 1), HYB294-06-02 (type 2), HYB300-06 (type 3) from Thermo Fisher Scientific, or 4-8-7 (Bilthoven Biologicals, Bilthoven, The Netherlands) were added and incubated for 2h at 37°C. After another washing step, HRP-labeled anti-mouse IgG (GE Healthcare, Buckinghamshire, UK) was added, plates were incubated for 1h at 37°C, washed, and SureBlue tetramethylbenzidine (TMB) Microwell Peroxidase Substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added. After 10 min, the reaction was stopped with 0.2 M sulfuric acid and absorbance at 450 nm was measured. Assay data were analyzed by four-parameter logistic curve fitting and D-antigen content was calculated relative to the reference standard.

Table 1 Design of Experiments worksheet. Composition of all formulations tested in a full factorial design were displayed in the design matrix.

Experiment No.	HPC (% w/v)	Glycerol (% w/v)	Sorbitol (% w/v)
N1	10	0	0
N2	15	0	0
N3	10	2.5	0
N4	15	2.5	0
N5	10	0	5
N6	15	0	5
N7	10	2.5	5
N8	15	2.5	5
N9	12.5	1.25	2.5
N10	12.5	1.25	2.5
N11	12.5	1.25	2.5

Design of Experiments (DoE)

A Design of Experiments (DoE) approach was used to evaluate the effects of HPC (10-15% w/v), glycerol (0-2.5% w/v) and sorbitol (0-5% w/v) on both D-antigen recovery upon drying and mechanical film properties. A full factorial screening design (Table 1) was performed and analyzed using Modde software (version 12, Umetrics AB, Umea, Sweden).

Models were fitted using multilinear regression (MLR) and subsequent optimized by deleting non-significant terms leading to a model with the best model performance parameters, i.e., goodness of fit (R^2), goodness of prediction (Q^2), model validity and reproducibility.

RESULTS AND DISCUSSION

Target product profile

The establishment of a target product profile (TPP) is a helpful tool to focus research on a certain technology and product development efforts in order to support efficient and directed product development [9]. A TPP describes the desired properties and characteristics of a final product. The current study was started with the establishment of a TPP for sIPV-containing orally dissolving film formulations appearing as (semi)-transparent films (Table 2). Moreover, some of the characteristics were defined as critical quality attribute (CQA), because these properties should be within an appropriate limit to ensure the desired product quality and thereby adequate performance and safety of the product when used [8]. The TPP is a dynamic summary that changes as knowledge during product development increases. Therefore, the anticipated quality target product profile (QTPP) might also be subjected to changes (Table 2).

Since the proposed administration route differs from the conventional route for sIPV (intramuscular or subcutaneous injection), dose-finding studies should define the single human dose for oral dosage forms, like oromucosal films. Moreover, the size of the film depends on what is acceptable for the intended target population and should thus be defined later in development (Table 2). Variability will affect efficacy, so content uniformity should be within certain limits, which will be defined upon selection of validated assays for the assessment of content and conform relevant guidelines. Moreover, a high or low pH will disrupt the polio particle thereby affecting the vaccine's efficacy. The pH limits were set on pH 6.5 - 7.0.

Unfortunately, mechanistic studies designed to evaluate and define the optimal conditions for sublingual (or buccal) vaccine delivery are lacking in literature. It remains speculative what, for example, the optimal release profile of an oral dosage form for sublingual vaccination is. Moreover, to what extend residual water content of oral films might affect product performance or stability is still unknown. Hence, CQA targets, but also critical process parameters having an impact on CQAs, should be determined during future product development and based

on risk assessments. Although they are relevant for the final product, it was decided not to focus extensively on all critical quality characteristics, like water content, dissolution and disintegration time, during the current (preliminary) study on sIPV-oral films.

Table 2 Target product profile of an IPV-containing oromucosal film. The current table summarizes the desired target product profile of an orally dissolvable film containing Sabin IPV.

Attributes	Target product profile (TPP)	Anticipated QTPP[1] - CQA[2]	Justification
Dosage form	Oromucosal film	No	n.a.
Appearance	(semi-)transparent film Size: t.b.d. Thickness: 5-200 µm	No	Target set to ensure recipient acceptability
Target population	Infants	No	
Administration route	Buccal or sublingual	No	
Dose	t.b.d.	Yes	Dose finding studies should define dose needed for buccal or sublingual route. *NB: Currently used (parenteral) sIPV dose: 10-16-32 DU.*
Content uniformity	t.b.d.	Yes	Variability will affect efficacy
Pharmaco-kinetics	Dissolution rate: t.b.d. Antigen release within 24h	Yes	Release profile is important for bioavailability and antigen uptake. Also recipient acceptability plays a role.
Storage	2-8°C	No	Defined based on stability results.
Stability	At least 36 months shelf-life at 2-8°C	Yes	Ideally, oral sIPV films should be at least as stable as the liquid sIPV vaccine. Depending on acceptable dose range (efficacy and safety).
Container closure system	t.b.d.	Yes	Needed to achieve target shelf-life and ensure the product integrity during storage. *NB: If defined properly without having impact on product quality and integrity, this attribute is not critical.*
pH	6.5 < pH < 7.0	Yes	High or low pH will disrupt antigen and affect efficacy.
Residual water content (RMC)	t.b.d.	Yes	Depending on design space, acceptable RMC range and subsequent stability and D-antigen recovery as result of formulation and drying process. *NB: Limited amounts of water in oral dosage forms will not impact patient safety or efficacy.*
Disintegration	t.b.d.	Yes	Film strength will affect release profile and may impact efficacy.

[1] Quality target product profile (QTPP) – prospective summary of the quality characteristics; critical quality attributes.
[2] Critical Quality Attribute (CQA) - should be within defined targets to ensure the desired product quality.
Abbreviations used: n.a. - not applicable; t.b.d. - to be determined

Table 3 Oral film formulations tested in full factorial screening experiment using a Design of Experiments (DoE) approach including hydroxypropyl cellulose (HPC), glycerol and sorbitol as excipients. Viscosity of the casting solutions was measured and dried oral films that were removable from the release liner were further characterized.

Exp. No.	Viscosity (mPa.s, 20 rpm)	Removable from release liner	Mass (mg)	Thickness (µm)	Residual moisture (%)	Elongation at break (%)	Young's Modulus (N/mm2)	Tensile strength (N/mm2)	Disintegration time (s)
N1	296	+	25.2 ± 2.3	61.9 ± 5.1	2.93	20.2 ± 7.9	315.5 ± 49.3	2.8 ± 0.6	16.2 ± 2.1
N2	1490	+	35.9 ± 2.0	86.4 ± 5.1	3.14	28.1 ± 6.9	319.4 ± 22.9	3.2 ± 0.3	29.0 ± 3.2
N3	270	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N4	1700	+/-	39.9 ± 3.4	91.4 ± 10.1	7.69	93.1 ± 35.6	71.2 ± 11.2	0.5 ± 0.2	19.7 ± 3.0
N5	458	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N6	2900	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N7	634	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N8	3170	+/-	48.7 ± 2.9	137.1 ± 27.4	8.73	17.1 ± 6.0	61.3 ± 25.4	0.6 ± 0.2	14.8 ± 2.7
N9	890	+/-	38.5 ± 5.1	101.5 ± 12.1	6.39	14.6 ± 3.7	93.9 ± 30.8	0.8 ± 0.3	14.4 ± 4.7

n.d. – not detectable

Table 4 Oral film formulations tested in full factorial screening design including hydroxypropyl cellulose (HPC), glycerol and sorbitol as excipients. D-antigen recoveries were determined per serotype directly upon drying.

Exp. No.	HPC (% w/v)	Glycerol (% w/v)	Sorbitol (% w/v)	D-antigen recovery (%)		
				Type 1	Type 2	Type 3
N1	10	0	0	9.1	n.d	10.8
N2	15	0	0	13.6	n.d	20.0
N3	10	2.5	0	n.d.	n.d	6.0
N4	15	2.5	0	n.d.	n.d	5.5
N5	10	0	5	48.2	32.8	34.7
N6	15	0	5	40.8	19.7	34.6
N7	10	2.5	5	30.3	42.9	36.8
N8	15	2.5	5	27.0	39.5	39.0
N9	12.5	1.25	2.5	7.8	22.2	27.0
N10	12.5	1.25	2.5	7.1	17.8	24.4
N11	12.5	1.25	2.5	7.5	16.2	24.5

n.d. – not detectable

Full factorial screening design

To gain insight into the effects of the main components of oral film formulations, i.e. film forming polymer hydroxypropyl cellulose (HPC), and plasticizers glycerol and sorbitol, on both physical/mechanical film characteristics and D-antigen recovery, a full factorial screening design was performed. Viscosity of the (placebo) casting solutions was measured and ranged from 270 to 634 mPa.s and from 1490 to 3170 mPa.s for formulations containing respectively 10% (w/v) (N1, N3, N5 and N7) and 15% (w/v) HPC (N2, N4, N6 and N8) (Table 3). All solutions were homogenous and no air bubbles occurred during the casting procedure, so all were suitable for casting. Formulation N1, consisting of 10% (w/v) HPC only, spreads too much directly after casting due to the relatively low viscosity, whereas appropriate spreading occurred upon casting of formulation N2. Formulations containing the lowest concentration HPC (10% (w/v)) displayed holes and were defined as unsuitable for further testing due to their handling difficulty, since these formulations yielded very thin and fragile films that, in the presence of sorbitol and/or glycerol, cannot be removed from the release liner (Table 3). The inclusion of sorbitol resulted in sticky films that were at least difficult or unable to subtract from the release liner, so these formulations (N5, N6 and N7 of table 3) were not further analyzed for mechanical film properties. The sorbitol-containing formulations that could be taken from the release liner upon drying consisting of 15% w/v HPC, 2.5% w/v glycerol and 5% w/v sorbitol (N8) or 12.5% HPC, 1.25% glycerol and 2.5% w/v/ sorbitol (N9).

Upon removal from the release liner, oral film formulations were further characterized for their physical (i.e., mass, thickness, residual water content) and mechanical properties (elongation at break, Young's Modulus, tensile strength and disintegration time). Increasing the concentration of excipients resulted in oral films with increased mass and thickness (Table 3). Moreover, addition of excipients resulted in higher percentages of residual water (defined as weight loss on drying) and negatively influenced the tensile strength (Table 3).

The oral films disintegrated all within 30 seconds (Table 3). This is compliant with guidelines for orally disintegrating tablets in European Pharmacopoeia (EP) and United States Pharmacopoeia (USP) (disintegration within 180 s) [11, 13, 14]. However, these recommendations might be less useful for our vaccine-containing films as antigen-transport through oral mucosa and subsequent uptake by immune cells might be time-consuming steps, which might need a prolonged residence time of the vaccine-formulation into the mouth. As described in section 3.1, it remains speculative what the optimal release profile of vaccine-containing oral films will be. As such, clearly defined requirements for disintegration time of oromucosal (fast or slow release) films have to be defined, subsequently by pharmaceutical guidelines.

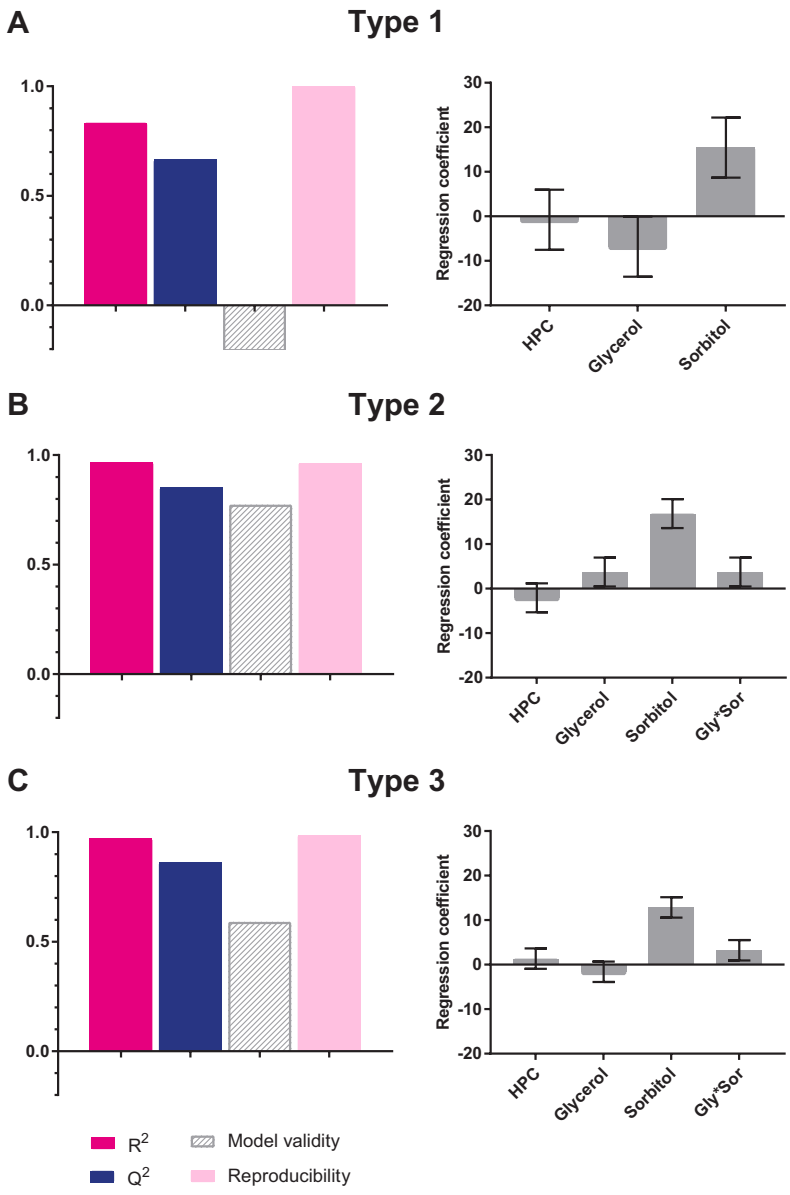

Figure 2 Regression models for the D-antigen recoveries type 1 (A), type 2 (B) and type 3 (C) of oral film formulations directly after drying. On the left, summaries of fit for all models are depicted: goodness of fit (R^2, values >0.5 indicate a good model), goodness of prediction (Q^2, values >0.5 indicate a good prediction power), model validity (values >0.25 indicate that the model is smaller than the experimental error) and reproducibility (values >0.5 indicates a small experimental error). On the right, main and interaction effects of the components (i.e., hydroxypropyl cellulose (HPC), sorbitol (Sor) and glycerol (Gly)) that contribute (per serotype) to the best fitted model, according to their model performance parameters are shown in model coefficient plots. Note: The negative model validity value for type 1 (A) here is a direct result of the extremely good replicates and a reproducibility value close to 1.

To evaluate the effect of the excipients on D-antigen recovery upon drying, all formulations from the screening design were prepared freshly wherein sIPV was mixed (Table 1). To avoid spreading of the liquid formulations with low viscosity and allow film casting using small volumes to minimize the amount of antigen needed, vaccine-containing casting solutions were converted in films using the ring-based casting method. Directly after drying, films were dissolved and D-antigenicity was determined by ELISA (Table 4). Multilinear regression (MLR) models were fitted using the ELISA results and optimized per serotype, which resulted in valid models for the prediction of D-antigen recoveries of dried sIPV-films according to the model performance parameters for type 1 (Figure 2A), type 2 (Figure 2B) and type 3 (Figure 2C). Model validity for the type 1 D-antigenicity was negative, which is likely a model artifact and a direct result of the very low variation in the replicates (N9-N11) resulting in a reproducibility value close to 1 [15]. In general, most of the D-antigen content was lost during the drying process as indicated by D-antigen recoveries of <50% for all serotypes with the highest D-antigen recoveries obtained in the presence of sorbitol in the formulation (N5-N9) (Table 4). The stabilizing effect of sorbitol was also confirmed by the significant positive regression coefficients of the prediction models for D-antigen recovery type 1, 2 and 3 directly after drying (Figure 2). The film forming polymer HPC did not influence D-antigen recovery, whereas the addition of glycerol seemed to affect type 1 D-antigenicity negatively (Figure 2A), but have a slight stabilizing effect on type 2 D-antigenicity during the drying process (Figure 2B). Both for type 2 and 3, a significant interaction effect was observed when glycerol and sorbitol were combined in the formulation (Figure 1B-C). Response contour

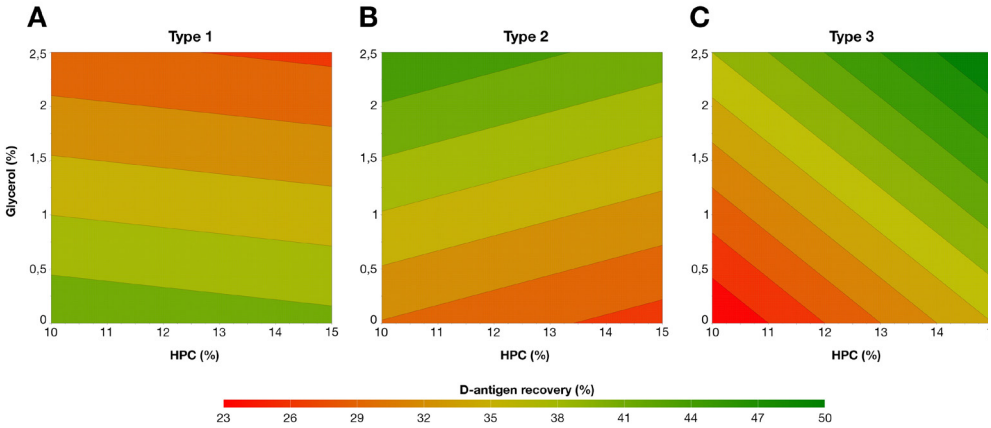

Figure 3 Response contour plots showed predicted D-antigen recoveries type 1 (A), type 2 (B) and type 3 (C) of formulations containing HPC (10-15% w/v, on x-axis) and glycerol (0-2.5% w/v, on y-axis) in combination with 5% (w/v) sorbitol.

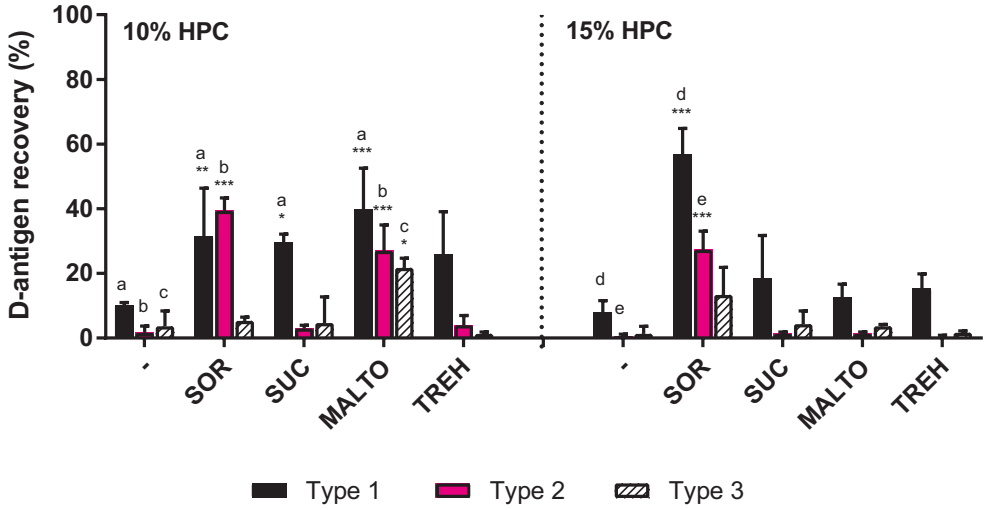

Figure 4 D-antigenicity of oral film formulations containing 10% (w/v) or 15% (w/v) hydroxypropyl cellulose (HPC) as film former and 5% (w/v) of sorbitol (SOR), sucrose (SUC), maltodextrin (MALTO) or trehalose (TREH). Directly upon drying, oral films were dissolved and D-antigen recoveries were determined for type 1 (black bars), type 2 (pink bars) and type 3 (striped bars) by ELISA. Mean values (n=3) and standard deviations are depicted. Asterisks indicate significant differences of formulations when compared with control formulation without additional sugars (-) marked with the same letter above bars (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

plots showed the challenge of developing a trivalent dried Sabin IPV since each serotype showed a different optimal combination of excipients (Figure 3). Earlier studies revealed the difficulty of drying IPV, which was based on Salk strains instead of the Sabin strains based IPV formulated here, using different drying methods [16-18]. Therefore, it was decided to focus on D-antigenicity, instead of film characteristics, in the upcoming experiments in order to improve vaccine's stability during the drying process.

Sugar screening

To increase the D-antigen recovery of the film formulations after drying, additional excipients may be included. Sugars have the ability to protect biologicals, like proteins or vaccines, against dehydration stresses [19]. The carbohydrates sucrose, maltodextrin and trehalose were screened in combination with 10% (w/v) or 15% (w/v) HPC. Since sorbitol already showed to confer protection during drying, sorbitol was also included. Glycerol was excluded from this experiment, since no clear positive effect on the D-antigen recovery of HPC-containing film formulations directly upon drying was found (data not shown). For formulations containing 10% HPC (w/v), both sorbitol, sucrose and maltodextrin conferred protection of sIPV serotype 1. However, only the addition of sorbitol or maltodextrin

significantly improved D-antigen recoveries of sIPV type 2, whereas only adding maltodextrin resulted in higher sIPV type 3 recoveries (Figure 4). Oral films consisting of 15% (w/v) HPC profited only from the inclusion of sorbitol as revealed by significant improved D-antigen recoveries for type 1 and 2 (Figure 4). These results indicated that a more complex formulation is needed to obtain an oral dissolvable film formulation while maintaining D-antigenicity. This is in contrast with literature showing D-antigen recoveries of more than 50% for all serotypes with vacuum-dried Salk IPV in combination with only one of the carbohydrates, which were also tested in the current study [16, 18]. However, the combination of sorbitol, magnesium chloride ($MgCl_2$) and monosodium glutamate (MSG) stabilized Salk IPV both during freeze-drying and vacuum-drying to a higher extent than a formulation with sorbitol only [16, 18].

Effect of $MgCl_2$ and MSG during drying

Certain excipients or combinations of excipients, like $MgCl_2$ and MSG, have the ability to drastically improve IPV stability during drying [16, 18]. In order to evaluate the effect of $MgCl_2$ and MSG in an oral film formulation, these excipients were tested in combination with sorbitol in HPC-films. Until now, highest type 3 D-antigen recoveries were obtained for the oral film consisting of 10% (w/v) HPC and 5% (w/v) maltodextrin, so this formulation was also tested in combination with $MgCl_2$ and MSG. Unfortunately, formulations consisting of 15% (w/v) HPC and 5% (w/v) of a sugar/polyol in combination with MSG did not result in homogeneous casting solutions, so only 10% (w/v) HPC-films were evaluated in this experiment. In agreement with previous work, the combination of sorbitol, $MgCl_2$ and MSG was able to protect the polio vaccine during drying as revealed by significant higher type 2 and type 3 D-antigen recoveries directly upon drying when compared with the control formulation consisting of only 10% (w/v) HPC and 5% (w/v) sorbitol (Figure 5). D-antigen recoveries of type 1 were significantly higher than those obtained with only $MgCl_2$ or MSG added to the control formulation. For type 3, the most vulnerable serotype, the combination of sorbitol, $MgCl_2$ and MSG resulted in a substantial increase in recovery after drying. Using the carbohydrate maltodextrin, as a substitute for sorbitol, did not result in improved stabilization, but showed instead a significant drop in the D-antigen recovery of type 3. This experiment showed the potential of a sIPV-containing oral film formulation with less than 25% loss in D-antigen content during drying. The formulation consisting of 10% (w/v) HPC, 5% (w/v) sorbitol, 3% (w/v) $MgCl_2$ and 3% (w/v) MSG resulted in an oral film with D-antigen recoveries of 110 ± 10% for type 1, 83 ± 7% for type 2, and 76 ± 3% for type 3.

However, although D-antigen recoveries were ideal or at least high, all sorbitol-containing formulations resulted in sticky films that were difficult to remove from the release liner and felt very fragile. It would be hard to apply such a formulation into the oral cavity. This may be solved by further matrix optimization with focus on better physical properties with remaining high antigen recovery. Alternatively, preparation of bilayered oral films might result in formulations with suitable film mechanical properties allowing easy handling and application [20, 21].

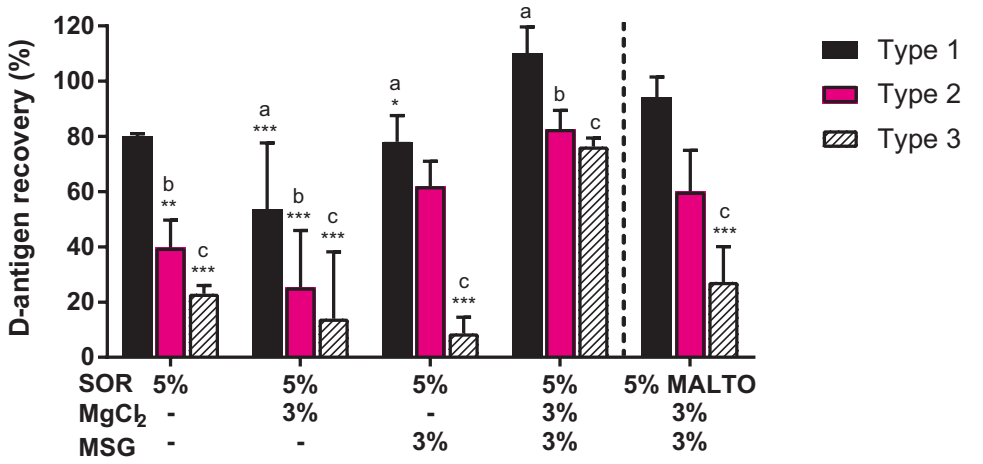

Figure 5 D-antigenicity of oral film formulations containing 10% (w/v) hydroxypropyl cellulose (HPC) as filmformer and 5% (w/v) of sorbitol (SOR) or maltodextrin (MALTO). The stabilizing effect of magnesium chloride ($MgCl_2$) and monosodium glutamate (MSG) was investigated. Directly upon drying, oral films were dissolved and D-antigen recoveries were determined for type 1 (black bars), type 2 (pink bars) and type 3 (striped bars) by ELISA. Mean values (n=3) and standard deviations are depicted. Asterisks indicate significant differences of formulation when compared with the formulation marked with the same letter above bars (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Sodium alginate and sodium carboxymethyl cellulose as film formers

Two polymers, i.e., sodium alginate (SA) and sodium carboxymethyl cellulose (CMC), were selected from literature and evaluated as film forming component in sIPV-containing oral films in order to try to improve film characteristics and maintaining high D-antigen recoveries. Preliminary experiments revealed that the addition of glycerol to SA-containing films resulted in more flexible films that can be easily removed from the release liner (data not shown). Therefore, oral film formulations comprising of 5% (w/v) SA, 5% (w/v) sorbitol and 1.25% glycerol were prepared and it was investigated whether the addition of both $MgCl_2$ and MSG was able to further improved D-antigen recoveries directly upon drying. In agreement with previous experiments with HPC as film former, the combination of sorbitol, $MgCl_2$ and MSG

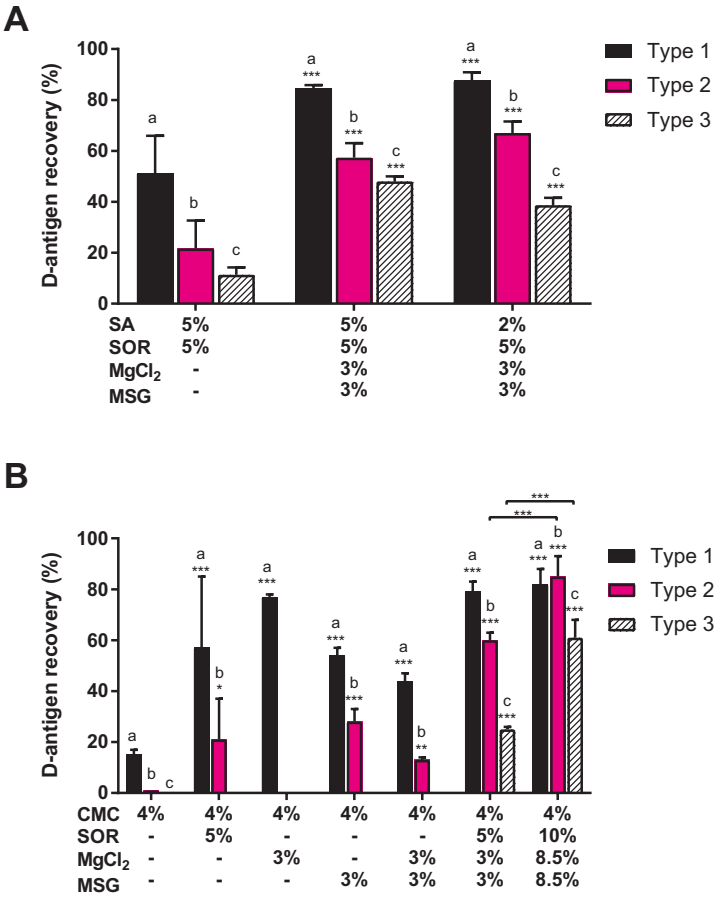

Figure 6 D-antigenicity of oral film formulations comprising of film formers sodium alginate (SA) (A) or sodium carboxymethyl cellulose (CMC) (B) as film former combined with a basic combination of sorbitol, magnesium chloride ($MgCl_2$) and monosodium glutamate (MSG). Directly upon drying, oral films were dissolved and D-antigen recoveries were determined for type 1 (black bars), type 2 (pink bars) and type 3 (striped bars) by ELISA. All SA-containing films containing also 1.25% (v/v) glycerol (A). Mean values (n=3) and standard deviations are depicted. Asterisks indicate significant differences of formulation when compared with the formulation marked with the same letter above bars (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

was able to protect the polio antigen during drying in an oral film formulation using SA as film former as exposed by significant higher D-antigen recoveries for all serotypes when compared to those obtained after drying the same formulation in the absence of $MgCl_2$ and MSG (Figure 6A). Using a lower concentration of film former, 2% (w/v) instead of 5% (w/v) SA, resulted in comparable D-antigen recoveries of 85-90% for type 1, 55-65% for type 2, and 40-50% for type 3.

Subsequently, the combination of sorbitol, $MgCl_2$ and MSG was evaluated in a formulation consisting 4% (w/v) CMC as film former. Besides, it was examined what the stabilizing effect was of each of these excipients was on the polio particle during drying. Directly upon drying, D-antigenicity of oral film formulations were assessed. Addition of each of the excipients (i.e., sorbitol, $MgCl_2$ and MSG) resulted in significant higher type 1 D-antigen recoveries, whereas the combination thereof resulted in significantly improved antigenicity of sIPV for all serotypes (**Figure 6B**). Type 3 showed again to be the most vulnerable serotype to stabilize during drying with detectable, but low recoveries (25 ± 1%) when using 5-3-3% (w/v) of respectively sorbitol, $MgCl_2$ and MSG. Addition of increasing amounts of the stabilizing components resulted in enhanced type 2 and type 3 D-antigen recoveries. The higher excipient concentrations (i.e., 10-8.5-8.5% (w/v) of respectively sorbitol, $MgCl_2$ and MSG) were based on the optimal formulation in our previous study on lyophilized IPV [16].

CONCLUDING REMARKS

The oral mucosa, especially the sublingual region, might be an attractive vaccine delivery site since it is densely populated with specialized dendritic cells while the adjacent submucosal tissue is drained by lymphatic vessels, through which free antigen as well as antigen-loaded dendritic cells can migrate to regional lymph nodes [4, 22]. However, although mucosal surfaces are the main route for pathogen entry, yet the induction of effective (mucosal) immunity elicited by vaccine antigens is a major challenge. Mechanistic studies designed to evaluate and define optimal conditions for sublingual or buccal vaccine delivery of macromolecules or even particles are still lacking in literature. Ideally, a minimal contact time of the dosage form for optimal antigen transport through oral mucosa should be defined. Also the question whether solid oral dosage forms would be preferred over liquid formulations are not answered. The risk of swallowing and/or salivary wash-out when administering liquid formulation to the oral mucosa exists, but liquid administration may also improve antigen uptake due to a larger contact area between vaccine and sublingual mucosa. Ideally, a head-to-head comparison is made between different oral films and (thermoresponsive) gel formulations with distinctive retention times to optimize mucosal contact area and contact time for sublingual delivery. Moreover, antigen transport through oral mucosa and uptake by immune cells could also be affected by muco-adhesive and penetration-enhancing components. The inclusion of such components in oral films might improve antigen delivery

via sublingual (or buccal) film application as well. Furthermore, for the induction of evident immunity (i.e., systemic and local mucosal immunity) upon vaccination under the tongue, inclusion of adjuvants may be required.

Aim of the current study was to evaluate the possibility to make polymer-based oral dissolvable films containing trivalent sIPV. In order to facilitate the formulation screening and optimization an antigen sparing ring-based film casting method was developed. Maintaining the antigenicity of the polio particle during film casting and drying showed to be challenging, especially for sIPV type 3. Addition of the excipients sorbitol, $MgCl_2$ and MSG protected the antigen during the drying process, irrespective of the film forming component that was used. This study yielded oral film formulations containing sIPV while preserving its D-antigenicity for 85-100% for type 1, 60-85% for type 2, and 50-75% for type 3. Amongst these sIPV film formulations, the sIPV film based on CMC-containing formulations showed highest D-antigen recoveries and is most appropriate for further product development in which several aspects need to be considered. The product profile of sIPV-containing oral films consisting of 4% (w/v) CMC, 10% (w/v) sorbitol, 8.5% (w/v) $MgCl_2$ and 8.5% (w/v) MSG gives a clear indication of the attributes, like stability, dissolution, disintegration and water content, that need to be evaluated during further development (**Table 5**). Due to shrinkage of the casting solution when using the standard casting method, it was not possible to obtain reproducible oral film formulations upon casting on a film casting apparatus. Since scalability of the production process is an important issue, further optimization of both formulation (e.g., inclusion of surfactant) and production process (e.g., release liner, casting speed) might be desirable. Moreover, based on future insights in the contribution of oral films to immunogenicity of sublingually administered sIPV, attention should be paid to further optimization of film characteristics, including mechanical properties for ease of handling and release kinetics.

Table 5 Product profile of sIPV-containing orodissolvable film consisting of 4% (w/v) sodium carboxymethyl cellulose, 10% sorbitol, 8.5% magnesium chloride and 8.5% (w/v) monosodium glutamate cast using the ring-based casting method. Mean values and standard deviation (n=3) are shown.

Attributes	Product profile (PP)	Target[1] achieved?
Dosage form	Orally dissolving film	Yes
Appearance	Transparent film Removable from release-liner	Yes Yes
Size Thickness	Ø 16 mm circle Not measured	Unknown Unknown
D-antigen recovery	82 ± 6% (type 1) 85 ± 8% (type 2) 61 ± 7% (type 3)	Unknown
Content uniformity Mass	Not measured 100 ± 2 mg	Unknown
Stability	Not measured	Unknown
Dissolution	Not measured	Unknown
Disintegration	Not measured	Unknown
pH	7.0 ± 0.1	Yes
Water content [2]	7.1 ± 1.8%	Unknown

[1] *For expected targets see target product profile (TPP) in table 2.*

[2] *Theoretical water content determined as percentage weight loss upon drying.*

REFERENCES

1. Kraan H, van der Stel W, Kersten G, Amorij JP. Alternative administration routes and delivery technologies for polio vaccines. Expert Rev Vaccines. 2016;15:1029-40.
2. Wright PF, Wieland-Alter W, Ilyushina NA, Hoen AG, Arita M, Boesch AW, *et al*. Intestinal immunity is a determinant of clearance of poliovirus after oral vaccination. J Infect Dis. 2014;209:1628-34.
3. Kraan H, Soema P, Amorij JP, Kersten G. Intranasal and sublingual delivery of inactivated polio vaccine. Vaccine. 2017;35:2647-53.
4. Kraan H, Vrieling H, Czerkinsky C, Jiskoot W, Kersten G, Amorij JP. Buccal and sublingual vaccine delivery. J Control Release. 2014;190:580-92.
5. White JA, Blum JS, Hosken NA, Marshak JO, Duncan L, Zhu C, *et al*. Serum and mucosal antibody responses to inactivated polio vaccine after sublingual immunization using a thermoresponsive gel delivery system. Human vaccines & immunotherapeutics. 2014;10:3611-21.
6. Lee BY, Cakouros BE, Assi TM, Connor DL, Welling J, Kone S, *et al*. The impact of making vaccines thermostable in Niger's vaccine supply chain. Vaccine. 2012;30:5637-43.
7. Some vaccine costs are hidden below the surface. Project Optimize - Ideal supply systems for the future. http://www.who.int/immunization/programmes_systems/supply_chain/optimize/resources/en/: WHO; PATH; 2012.
8. Rathore AS, Winkle H. Quality by design for biopharmaceuticals. Nat Biotechnol. 2009;27:26-34.
9. van der Pol L, Amorij J.-P. Vaccine Development: History, Current Status, and Future Trends. Pharmaceutical Sciences Encyclopedia 2013. p. 1-26.
10. Thomassen YE, Rubingh O, Wijffels RH, van der Pol LA, Bakker WA. Improved poliovirus D-antigen yields by application of different Vero cell cultivation methods. Vaccine. 2014;32:2782-8.
11. Visser JC, Woerdenbag HJ, Crediet S, Gerrits E, Lesschen MA, Hinrichs WLJ, *et al*. Orodispersible films in individualized pharmacotherapy: The development of a formulation for pharmacy preparations. Int J Pharm. 2015;478:155-63.
12. Westdijk J, Brugmans D, Martin J, van't Oever A, Bakker WA, Levels L, *et al*. Characterization and standardization of Sabin based inactivated polio vaccine: proposal for a new antigen unit for inactivated polio vaccines. Vaccine. 2011;29:3390-7.
13. Hoffmann EM, Breitenbach A, Breitkreutz J. Advances in orodispersible films for drug delivery. Expert Opin Drug Deliv. 2011;8:299-316.
14. Preis M, Woertz C, Kleinebudde P, Breitkreutz J. Oromucosal film preparations: classification and characterization methods. Expert Opin Drug Deliv. 2013;10:1303-17.
15. Eriksson L, Johansson E, Kettaneh-Wold N, Wikström C, Wold S. Design of Experiments; Principles and Applications: Umetrics AB; 2008.
16. Kraan H, van Herpen P, Kersten G, Amorij JP. Development of thermostable lyophilized inactivated polio vaccine. Pharmaceutical research. 2014;31:2618-29.
17. Edens C, Dybdahl-Sissoko NC, Weldon WC, Oberste MS, Prausnitz MR. Inactivated polio vaccination using a microneedle patch is immunogenic in the rhesus macaque. Vaccine. 2015;33:4683-90.
18. Tzeng SY, Guarecuco R, McHugh KJ, Rose S, Rosenberg EM, Zeng Y, *et al*. Thermostabilization of inactivated polio vaccine in PLGA-based microspheres for pulsatile release. J Control Release. 2016;233:101-13.
19. Wang W. Lyophilization and development of solid protein pharmaceuticals. Int J Pharm. 2000;203:1-60.
20. Preis M, Woertz C, Schneider K, Kukawka J, Broscheit J, Roewer N, *et al*. Design and evaluation of bilayered buccal film preparations for local administration of lidocaine hydrochloride. Eur J Pharm Biopharm. 2014;86:552-61.
21. Visser JC, Weggemans OAF, Boosman RJ, Loos KU, Frijlink HW, Woerdenbag HJ. Increased drug load and polymer compatibility of bilayered orodispersible films. Eur J Pharm Sci. 2017;107:183-90.
22. Masek J, Lubasova D, Lukac R, Turanek-Knotigova P, Kulich P, Plockova J, *et al*. Multi-layered nanofibrous mucoadhesive films for buccal and sublingual administration of drug-delivery and vaccination nanoparticles - important step towards effective mucosal vaccines. J Control Release. 2016.