Stability of a Respirable Dry Powder Vaccine

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Summary

Using ovalbumin as a model antigen, the aim of this project was to develop a dry powder vaccine containing antigen-loaded nanoparticles which is stable at room temperature and suitable for delivery to the lungs. Chitosan nanoparticles were produced via ionic gelation whereby ovalbumin was encapsulated. Subsequently, the resulting nanosuspension was spray dried with the addition of mannitol as an immediately soluble matrix to transfer the nanosuspension into a dry respirable powder. Directly after manufacturing the powder was characterised with respect to redispersion behaviour and resulting particle size of the nanosuspension. Antigen loading and integrity was determined to confirm that the protein was successfully incorporated. Particle size distribution of the dry powder was characterised by laser diffraction to be 2.5 µm (x_{50}). The powder could be dispersed efficiently using the Cyclohaler as a model device. To determine long term stability the samples were stored at different conditions (refrigerated and at 25°C/60%rH) for up to 6 months. At predefined time points, samples were taken and analysed. It was shown that after storage time there were no major changes detectable in formulation, independent from storage conditions.

Introduction

During the last decade interest in particulate systems for vaccination has grown [1]. Especially the respiratory tract, with its presence of many immune cells and its highly vasculated, easy accessible tissue, seems to be appropriate for immunological applications [2]. Smaller particles in the size between 100 - 500 nm are taken up efficiently by antigen presenting cells and cause a higher IgG and IgA response compared to larger ones (>1000 nm) [3]. As antigen delivery system, chitosan nanoparticles are good antigen carriers besides showing good biocompatibility and biodegradability in addition to adjuvant and mucoadhesive effects [4]. However, not the nanoparticles themselves, but particles having an aerodynamic size of 0.5 µm – 5 µm are optimal for deposition in the respiratory tract. Such a powder is producible via spray drying. This is a fast continuous process and leads to fine spherical shaped particles, which show perfect properties for inhalation [5]. In this work ovalbumin as model antigen is incorporated in chitosan nanoparticles, which are then spray dried with a matrix to obtain a formulation for pulmonary delivery. In the present study, dispersion characteristics and storage stability are evaluated.

Materials and Methods

Preparation of the formulation

Nanoparticles were produced by ionic gelation. For this, 0.1% (w/v) chitosan (Heppe Medical Chitosan GmbH, Germany) and 1 mg/mL ovalbumin (OVA; Sigma, USA) were dissolved in 2% (v/v) acetic acid. 0.1% (w/v) of a negatively charged cellulose derivative (Tylose C30, Hoechst, Germany) as agent for ionic gelation was dissolved separately in double distilled water of the same volume and added slowly to the chitosan phase while stirring. 2% (w/V) mannitol (Pearlitol 200 SD, Roquette, France) was dissolved in the resulting nanosuspension and the preparation was spray dried using the Büchi B-290 Mini spray dryer (Büchi, Flawil, Switzerland) at an inlet and outlet temperature of 80°C and 35°C, respectively.

Storage conditions

Dried samples were packed in glass vials with a rubber stopper and crimping closure and were stored at 25°C/60 rH or in refrigerator (2°C - 8°C, ambient humidity). Samples were analysed after predefined time points (after manufacturing, after 1 month, 3 months, 6 months). The chosen target values are: Changes in primary size (nanoparticle size after redispersion of the powder), dispersibility of the powder and changes in particle size distribution of the dried powder and stability of antigen.

Particle size in suspension

Nanoparticle size was determined via photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern Instruments, UK). The initial nanosuspension was measured undiluted; for measurement of the resulting particle size after drying procedure 21 mg dry powder were redispersed in 1 ml 1% acetic acid. After an equilibration time of 2 minutes the sample undergoes a measurement of 15 runs which are averaged to one result. Data shows average of three measurements.

Particle size distribution of the dry powder

The dry powder was analysed by laser diffraction (Helos, Sympatec GmbH, Clausthal-Zellerfeld, Germany) upon dry dispersion at 3 bar. x_{50} (mean particle size) was calculated. Further, inhaler dispersion capability was tested with the

Cyclohaler, a dry powder inhaler device (DPI), representatively. For this 10 mg of powder were weighed individually into HPMC capsules. The capsule was pierced in the device and dispersed using the Inhaler module (Sympatec GmbH, Clausthal-Zellerfeld, Germany) at an airflow of 100 L/min corresponding to a pressure drop of 4 kPa (Ph.Eur.). Data shows average of three measurements. To compare the deagglomeration effectivity, the relative deagglomeration (rDeaggl) was calculated utilising Equation 1.

Equation 1

$$\mathrm{rDeaggl}_{\mathrm{Cyclohaler}} = \frac{\Sigma\ \mathrm{particles} < 5.25\ \mu\mathrm{m\ after\ dispersion\ Cyclohaler}}{\Sigma \mathrm{particles} < 5.25\ \mu\mathrm{m\ after\ dispersion\ at\ 3\ bar}}$$

Protein integrity

For protein integrity the molecular weight was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). 20 mg of dried powder were dissolved in 100 µl 10 x PBS 7.4 (Ph. Eur. 7.4) with a 1% addition of citric acid to fully release the protein. The samples were incubated for 5 minutes at 90°C in loading buffer (mercapto ethanol and SDS).10 µl per sample were loaded into the gel and electrophoresis was performed at a voltage of 120 V for 5 minutes for preseparation and then at 200 V for about 50 min. Afterwards the protein was stained using Coomassie Brilliant Blue (Carl Roth, Karlsruhe, Germany). Molecular weight (MW) was determined by comparison with a MW marker (PAGERuler^{TM},Thermo Scientific, Waltham, MA, USA).

Results and Discussion

During the ionic gelation process, particles with narrow size distribution (PDI 0.178 +/- 0.02) are formed spontaneously. The protein loading is around 10% (w/w) with a loading efficiency of 20% (w/w). Particle size of the nanosuspension is reproducible (209 nm +/- 16 nm) and independent from batch size (5 ml - 1000 ml). After spray drying the particles are easily redispersible and there is almost no change in size upon drying (Figure 1).

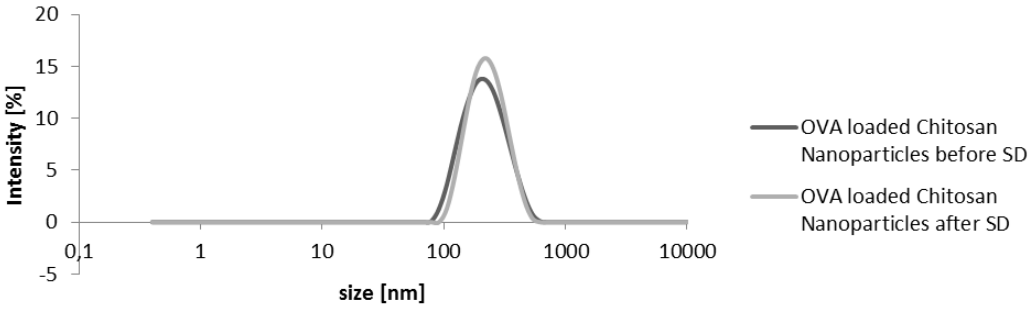

Figure 1: Nanoparticle size directly after preparation. Before SD (black line) and after SD and redispersion in 1% HAc (grey line) (SD: spray drying)

Nanoparticle size slightly increases within the first month of storage, for later time points there is almost no further change in mean particle size neither for refrigerated samples nor for the samples stored at 25°C/60 %rH (2-8°C: 243.9 nm +/- 9.2 nm (PDI 0.172 +/-0.004); 25°C/60%rH: 279.2 nm +/- 4.8 nm (PDI 0.245 +/- 0.015)). An increase of agglomerates can be observed in the latter case, indicated by the rised PDI (Figure 2). Despite a broader distribution, mean particle size remains in the required range for cellular uptake [3],[5].

SDS-PAGE shows no fragmentation of the protein in the tested 6 months range (Figure 3). This indicates that the primary structure of the protein has not been damaged by the manufacturing method, during storage at different conditions or upon release from the nanoparticles.

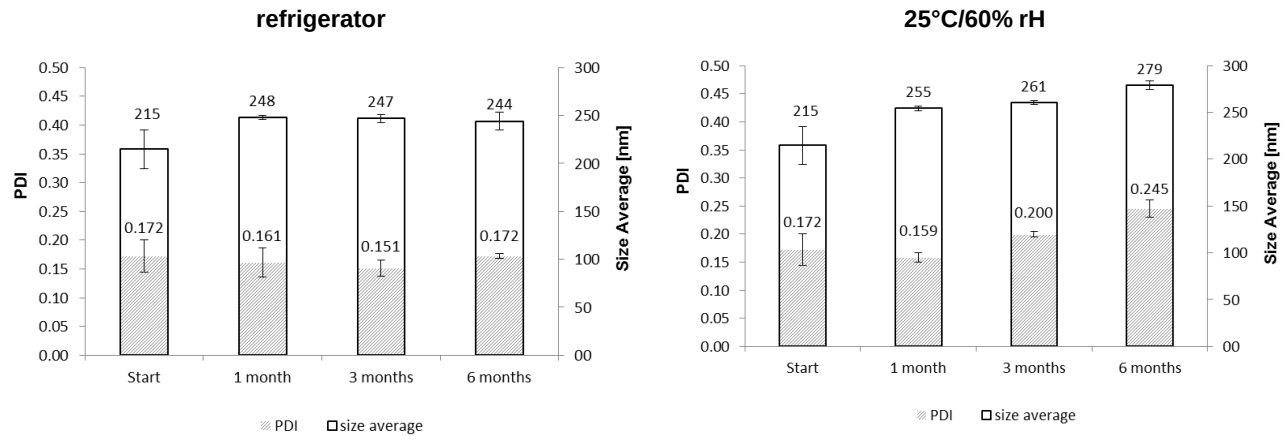

Figure 2: Nanoparticle size and PDI over storage. <u>Left</u>: size change for samples stored in refrigerator at ambient humidity; <u>right</u>: size change for samples stored at 25°C/ 60% rH.

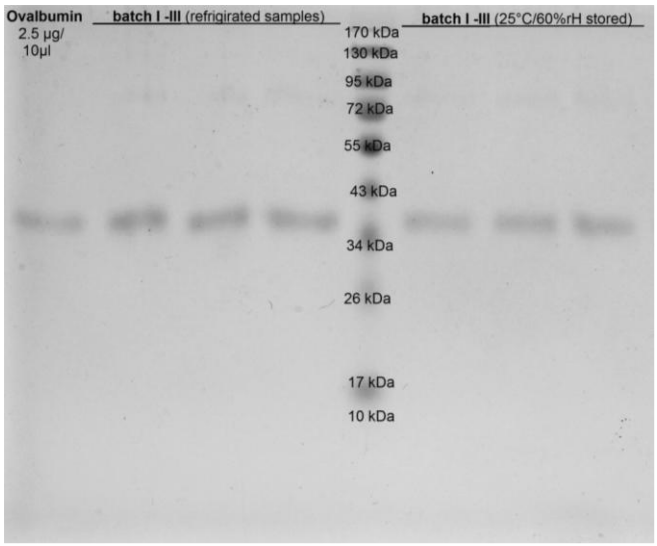

Figure 3: SDS-PAGE after 6 month of storage. Lines from left to right: OVA pure, OVA from refrigerated stored samples (3 batches), MW marker, OVA from 25°C/60%rH stored samples (3 batches).

Possible instabilities are not limited to the primary structure of a protein antigen, but may also affect proteins' secondary and tertiary structure leading to conformational changes. This can be fatal if the epitope is based on the antigen's supramolecular structure, where a discontinuous epitope is distributed on distant parts of the protein chain and is only building one location to be recognised due to protein folding. In this case, structure stabilisation is inevitable to maintain antigenicity. If the epitope is a continuous epitope within the protein chain, structure stabilisation is not necessary as only the epitope domain of the primary structure must remain intact [7]. For ovalbumin it has been reported that amino acids 257-264 (SIINFEKL) are responsible for immunologic effects [8]; as this octapeptide is encoded by a continuous DNA section a loss of antigenicity is not to be expected.

After spray drying, particle size measurements gave an x_{50} value of 2.55 µm (Figure 4). The particle size fraction below 5.25 µm is 83.4 % (theoretical fine particle fraction (FPF)). Using the Cyclohaler the relative deagglomeration (rDeaggl) based on the particle size distribution upon dispersion in pressurised air was 92.3% (Equation 1), which shows that the powder can be efficiently dispersed using that device. Upon storage there was no change in particle size distribution in the powder, regardless from storage conditions. A slight decrease in the fraction of particles below 5.25 µm upon degglomeration may be due to measurement circumstances. The capsules tended to get stuck due to electrostatic effects in the device after storage, so the rotation of the capsule was delayed.

Results show that the powder dispersion characteristics upon storage are promising for utilising this formulation for the delivery of antigen-carrying nanoparticles to the lung. Detailed characterisation of aerodynamic behaviour and immunological impact are ongoing.

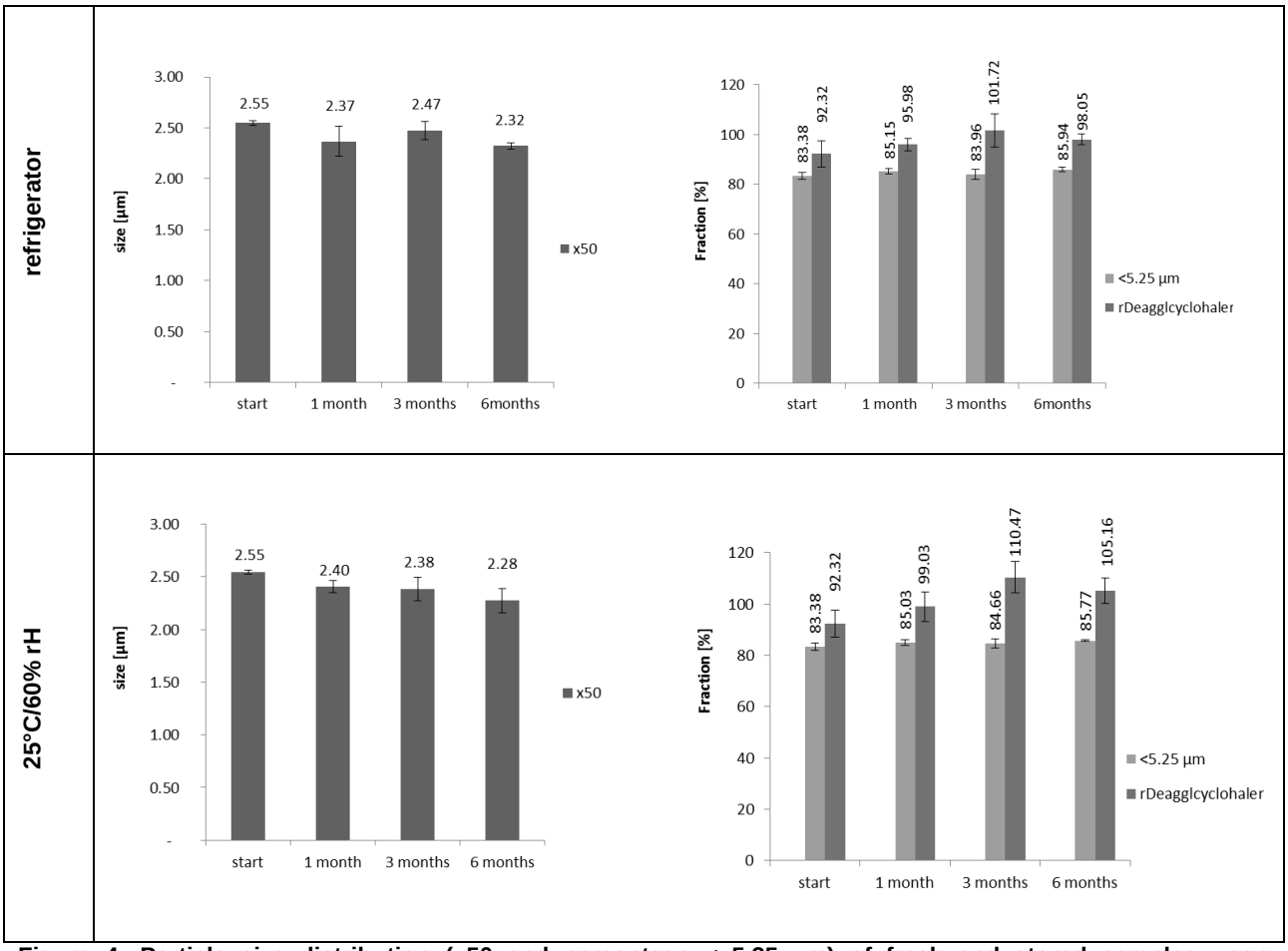

Figure 4: Particle size distribution (x50 and percentage < 5.25 µm) of fresh and stored samples upon dispersion at 3 bar air pressure (start, 1 month, 3 months, 6 months) and dispersion characteristics from the Cyclohaler. top: samples stored in refrigerator at ambient humidity; bottom: samples stored at 25°C/ 60% rH

Conclusion

Nanoparticles from chitosan with antigen are easy producible via ionic gelation. No change in primary size was observed after spray drying and redispersion. Mannitol being added to the nanosuspension before drying forms a carbohydrate matrix stabilising the individual nanoparticles. It also allows the formation of particles capable for direct dispersion from a DPI. The powder properties can be retained over storage at ambient conditions, which makes this a promising formulation for novel respiratory vaccines.

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