Combining Particle Engineering with Device Development to fine tune DPI performance of AP301

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Summary

AP301 is a new synthetic peptide designed for the treatment of pulmonary lung oedema through the activation of the ENaC channel of type II alveolar cells of the lungs. This product was already administered clinically in the form of a nebulized version, with very promising results in the treatment of patients suffering from Acute Respiratory Distress Syndrome and with primary graft dysfunction following lung transplantation. In order to further expand the range of applications for this product, and given that nebulization is not the preferred delivery route for the vast majority of at home patients, a dry powder inhalation platform was attempted for this product.

For that purpose a spray drying (SD) process was developed in order to produce different Drug Alone powders targeting different Particle Size Distributions (PSD). The objective was to manipulate this property of the powder in order to reduce cohesive / adhesive forces and thus to increase the aerodynamic performance. Throughout the program it was demonstrated that the SD process enabled obtaining stable materials, within the targeted PSD and with a high degree of purity and bioactivity.

Due to the rescue nature of the treatment in some of the intended applications, and due to the difficulty of handling capsule-based devices in emergency situations, the need for patients to have a highly portable and easy to use disposable device was identified. For this reason, a device development program was conducted, targeting the development of an enhanced version of the currently marketed single dose disposable device TwinCaps®, able to accommodate large payloads of spray dried powder. Prototypes of this device and one off the shelf device were used to determine the aerodynamic performance (APSD) of the produced powders. The results indicated the feasibility of obtaining high emitted and fine particle dosages for drug alone powders of AP301, with both types of devices, and that this performance remained stable throughout a 3 month period at different storage conditions (25ºC/60%RH and 40ºC/75%RH).

Introduction

The synthetic protein AP301 (*Solnatide*) is a new high dosage compound, designed to activate the ENaC chanel of type II alveolar cells of the lungs, and thus to clear pulmonary oedema arising from high altitude exposure, blood transfusions, lung infections (such as pneumonia and Influenza), and others [1]. Liquid aerosol formulations of *Solnatide* have already been developed and clinically tested, with success, in patients with pulmonary permeability oedema and Acute Respiratory Distress Syndrome and with primary graft dysfunction following lung transplantation [1]. However, in order to expand the ranges of application of this compound to a wider range of pulmonary indications, the development of a dry powder formulation (DPI) was attempted for AP301.

With the development program, particle engineering activities were pursued in order to develop a drug alone product (i.e. free of additional excipients) by spray drying (SD). This technology is well suited for proteins since, by promoting flash drying it prevents product denaturation[2]. Additionally, SD allows the production of free flowing powders with a particle size distribution within the inhalable range and with a well-controlled morphology[2]. The objective was to target different particle size distributions (PSD) in an attempt to reduce the adhesive / cohesive forces that are typically present in this type of products.

Two different devices were evaluated throughout the program, one off the shelf commercial device and the new disposable device, under development, from Hovione, the here designated, TwinMax™ concept. This device is an enhanced version of the disposable device TwinCaps® (initially developed for Laninamivir® carrier based formulation), aiming at the delivery of high dosages of challenging inhalations powders (for instance spray dried powders, which are typically characterized by having a low density and high cohesiveness). The TwinMax™ concept considers changes in terms of cavity size but also in the intrinsic dispersion / deagglomeration mechanisms; additional air vents were introduced in the shuttle of the device, to increase dispersive forces, and different deagglomeration mechanisms were explored via optimization of the shuttle outlet design (see figure 1)[3].

The best combinations of device / SD product were, at the end of the program, placed in stability under accelerated and non-accelerated conditions. The goal was to assess the impact of these conditions in the physical properties of the product (in terms of PSD, water content and purity) and also in the aerodynamic performance.

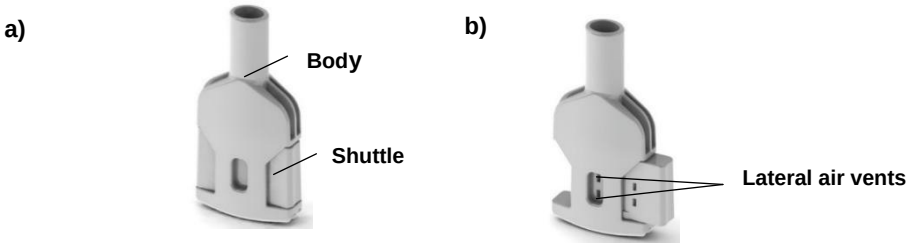


Figure 1. The TwinMax™ concept: a) Perspective view with shuttle in storage position; b) Perspective view with shuttle in actuation position showing additional lateral air vents into the powder compartment.

Materials and methods

❑ Spray Drying

Particle Engineering was performed by spray drying, using a Büchi unit model B-290 Advanced, with the API (AP301) dissolved in water. Different atomization ratios were targeted in order to fine tune the particle size distribution and to reduce the adhesive / cohesive balance in between particles and with the surfaces of the devices. Each produced batch was characterized in terms of PSD (by laser diffraction), of purity (by High Pressure Liquid Chromatography, HPLC), solid state form (by X-Ray powder diffraction, XRPD, and Modulated differential scanning calorimetry, mDSC), water content (by Karl Fisher determination, KF), hygroscopicity (by Dynamic Vapor sorption, DVS), morphology (by Scanning Electron Microscopy, SEM) and bioactivity.

❑ APSD evaluation by cascade impaction

The obtained products were characterized, in terms of their aerodynamic performance, using one off the shelf capsule based device (Plastiape, model RS01 operating at 60 L/min at 4 kPa) and three prototypes of the new TwinMax™ disposable device, developed by Hovione (TwinMax1 to 3, all operating at ~40 L/min at 4 kPa). The HPMC size #3 capsules (in the case of Plastiape) and shuttles of the devices (in the case of TwinMax™) were manually filled, with a fill weight of 50mg per capsule or per cavity (when using TwinMax™), with a 1% w/w tolerance over the fill weight, inside a glove box with environmental control (T<25ºC and %HR <15%). Gravimetric impaction (ACI) was used for the determination of the APSD profile and key aerodynamic parameters (Emitted mass (EM), Fine Particle Mass (FPM), and Fine Particle Fraction (FPF)). A total of 3 replicates were carried-out in order to characterize each combination of powder / device. When using the Plastiape device, the 60 L/min modified ACI method was used (stage 7 removed and stages -1 and -0 included), in order to comply with USP standards. With the TwinMax™ devices, the non-modified method was adopted given the similarity between tested flowrate and calibration flowrate. The mass deposited at each stage of the ACI (covered with a glass fiber filter) was determined gravimetrically, given that the product consisted of pure API.

❑ Short-termStability studies

Powder, obtained with the most promising SD conditions, was filled into HPMC size #3 capsules and into the prototype TwinMax™ devices, inside a glove box with environmental control (T<25ºC and %HR <15%).The samples were afterwards packed inside aluminum foils and placed in stability under accelerated (40ºC/75% RH) and non-accelerated conditions (25ºC, 60%RH). The testing scheme and the analytical tests performed in each timepoint are depicted in Table 1.

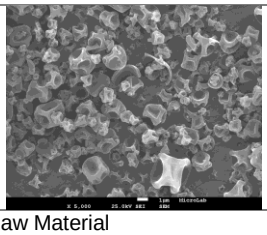
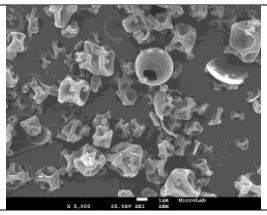
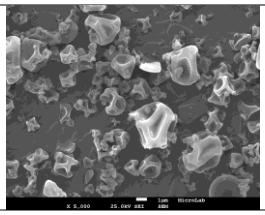
Table 1 - Testing scheme during the informal stability studies.

Condition /Timepoint	2 weeks	1 month	3 month
40ºC / 75% RH	HPLC, PSD, KF and ACI	HPLC, PSD, KF and ACI	-
25ºC / 60% RH	HPLC, PSD, KF and ACI	HPLC, PSD, KF and ACI	HPLC, PSD, KF and ACI

Results and discussion

Three spray drying runs were carried out targeting different particle size distributions (PSD). The SD parameter which was manipulated, in order to obtain these different PSD, was the ratio of atomization (i.e. the ratio between the solution feed flowrate and the atomization gas flowrate). All the other parameters, inlet and outlet temperature, solution feed flowrate and drying gas flowrate were kept constant throughout the batches. The objective was to determine the impact of PSD on the overall cohesiveness / adhesiveness of the produced powders and, consequently, on their aerodynamic performance. Each batch was characterized by HPLC, XRPD, mDSC, KF, DVS, SEM and bioactivity. The SD conditions and the obtained results are presented in Table 2.

Table 2 – SD conditions and analytical characterization of each manufactured batch

	Batch 1	Batch 2	Batch 3
Atomization Ratio	**$AR_{initial}$**	**0.65 x $AR_{initial}$**	**0.80 x $AR_{initial}$**
PSD (Dv10; Dv50; Dv90) (µm)	**0.9; 2.3; 3.9**	**0.9; 3.0; 5.9**	**0.8; 2.5; 4.9**
DVS (water gain @90% RH) (%)	14.4	14.5	14.2
KF (%)	4.9	3.4	3.9
Tg (ºC)	145	ND	ND
XRPD	Amorphous	Amorphous	Amorphous
Purity (by HPLC) (%)	97.3	97.3	97.3
Bioactivity	Same as SRM	Same as SRM	Same as SRM
Morphology (by SEM) (x5000, 25.0kV)			

ND – Not detected. SRM – Starting Raw Material

The obtained results showed that the manipulation of the ratio of atomization allowed a fine control of the PSD (with all Dv50 values comprised between 2 and 3µm). Additionally, all final powders were found to be amorphous, with a very high purity and with a low water content (KF < 5%). It was also observed that the powders were, as expected, very hygroscopic. In terms of bioactivity, all powders presented high bioactivities, suggesting that the SD process was not leading to degradation nor loss of function and, therefore, that no additional formulation work was necessary.

Finally, and in order to evaluate the impact of PSD manipulation on the final aerodynamic performance of the powders, gravimetric ACI testing was conducted. For that purpose, one off the shelf capsule based device (Plastiape) and three prototypes of TwinMax™ were considered (TwinMax1, TwinMax2, TwinMax3). The obtained results are depicted in Figure 2, for all powders / devices combinations.

(A)	**Batch 1**		**Batch 2**		**Batch 3**	
Parameters / Device	Plastiape	TwinMax1	Plastiape	TwinMax1	Plastiape	TwinMax1
EM / fill weight (%) (RSD %)	84.1 (3)	64.5 (11)	93.1 (2)	90.6 (3)	90.4 (2)	80.1 (6)
$FPF_{5µm}$/EM (%) (RSD %)	64.6 (1)	32.8 (9)	49.9 (18)	28.7 (10)	47.6 (18)	38.8 (5)

(B)

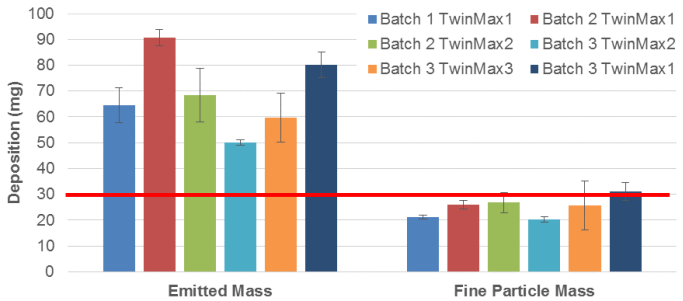


Figure 2 – (A) Aerodynamic performance of the 3 batches when tested with Plastiape and TwinMax1; (B) Comparison on the aerodynamic performance of several SD powders with different TwinMax prototypes (TwinMax1, TwinMax 2 and TwinMax 3).

The results suggested that the strategy of improving the aerodynamic performance, through the manipulation of the PSD of the powders, was successful. The combination of TwinMax1 with the Batch 3, with a Dv50 of 2.5µm, yielded a EM of 80% (15% increase when compared with Batch 1) and a FPF of 39% (6% increase when compared with Batch 1). An improvement in the EM when using the Plastiape device was also observed in Batch 3 (from 84% in Batch 1 to 91% in Batch 3). Overall, these results suggest that a compromise between PSD, EM and FPM needs to be considered, since higher PSD lead to higher EM but, in general, to lower FPM – consequence of a different balance between cohesive and adhesive forces.

The prototype which presented the most promising results was TwinMax1; although with lower FPM when compared with the Plastiape device, two points should be emphasized: i) TwinMax1 enabled FPM above 30 mg, a very impressive result considering the prototype nature of the device and ii) given the rescue / emergency nature of the treatment in some of the intended applications, providing a highly portable, easy to use, pre-filled disposable device as TwinMax™,will offer a number of important advantages over a capsule based device (as Plastiape).

A replicate of Batch 3 was made in order to place material in stability. The results of Batch 4 showed that the process was very consistent and reproducible, both in terms of physical/chemical properties and in terms of aerodynamic performance with the different devices (Figure 3 shows the full aerodynamic deposition profile with TwinMax1).

	Batch 3		Batch 4	
Parameters / Device	Plastiape	TwinMax1	Plastiape	TwinMax1
EM / fill weight (%) (RSD%)	90.4 (2)	80.1 (6)	87.2 (1)	84.8 (6)
$FPF_{6\mu m}$/EM (%) (RSD %)	47.6 (18)	38.8 (5)	54.2 (7)	32.7 (9)

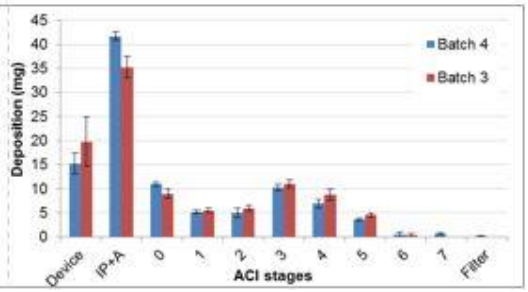


Figure 3 – Aerodynamic performance results of two replicate batches, when tested with TwinMax1 and with Plastiape.

The stability results obtained throughout the three month period indicate that the physical / chemical properties, the bioactivity and the aerodynamic performance was stable throughout the study, at the two different conditions (as can be observed in Figure 4 that shows the results obtained for Plastiape).

Capsules	Purity	Dv50	Bioactivity
T0	97.35	2.680	Same as SRM
T=2 w 25ºC/60% RH	96.95	2.726	Same as SRM
T=2 w 40ºC/75% RH	96.69	2.773	Same as SRM
T=1M 25ºC/60% RH	96.19	2.672	Same as SRM
T=1M 40ºC/75% RH	85.82	2.633	Same as SRM
T=3M 25ºC/60% RH	96.25	2.686	Same as SRM

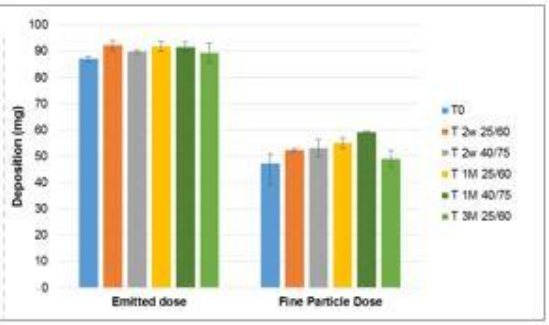


Figure 4 – Physico / chemical and aerodynamic performance results obtained throughout the stability program when using the Plastiape device.

Conclusions

Throughout this work, it was possible to obtain a stable spray dried powder of protein AP301, which complied, using both the off the shelf device and the new disposable TwinMax™ device, with the aerodynamic performance targets of EM > 80% and an FPF >30% (for a fill weight of 50 mg per cavity or per capsule). Additionally it was demonstrated that AP301's bioactivity was unaffected by the spray drying process and that the product remained stable and active throughout the stability program, at two storage conditions.

References

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