Pulmonary combination drug powders coated with selected amino acids

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Summary
Combination drug microparticles of beclomethasone and salbutamol sulphate were encapsulated and coated with amino acids L-leucine, L-valine and L-phenylalanine in the gas-phase in an aerosol reactor. The aim was to combine opposingly soluble drug materials into single particles and also to explore the influence of amino acid surface composition and texture on particle morphology and fine powder aerosolization. The amino acid coating was employed by partial vapor deposition on drug particle surfaces. Carrier-free powder aerosolization was studied using two different types of inhalers, Twister ™ and Easyhaler® at two pressure drops, 2 kPa and 4 kPa, over the inhalers. The powder emissions from the inhalers were relatively good but the fine particle fraction (FPF) depended very much on particle integrity and sintering degree. The best results were obtained with the leucine coated samples when the particles were well separated whereas the worst results, particularly the FPF, was obtained with the phenylalanine coated samples due to a strong particle sintering i.e. fusing between particles. Moreover, the leucine rough coating performed the best aerosolization properties in terms of emission and fine particle deposition and also independency of applied pressure drop inhalation flow rate.

Introduction
Combination therapies contain more than one drug to improve therapeutic outcomes via synergy action. This form of therapy has been used in the treatment of lung diseases such as pulmonary arterial hypertension (PAH) [1], asthma and chronic obstructive pulmonary disease (COPD) [2,3]. We demonstrate an aerosol-based gas-phase method to combine a water-soluble β-agonist drug, salbutamol sulphate, and corticosteroid, beclomethasone dipropionate, into fine particles which are subsequently encapsulated and coated with different amino acids, L-leucine, L-valine and L-phenylalanine. The aims were to explore the influence of amino acids on the aerosolization of fine drug powders using different inhalation flow rates and pressure drops over capsule and reservoir-based inhalers.

Materials and methods
Beclomethasone dipropionate (BP; a gift from Orion Pharma) suspensions (particle size 300-500 nm) were prepared using a wet-milling technique at 1100 rpm and 10 grinding/cooling cycles in a planetary milling machine (Pulverisette 7 Premium, Fritsch Co., Germany). Briefly, 1 g of Pluronic F68 (BASF) was dissolved in 10 ml of water followed by the 4 g of BP. The BP nanosuspension was added to a solution of salbutamol sulphate (S; Alfa Aesar) containing 0.8 g/l of bulking material D-mannitol (M; Alfa Aesar) and 20 g/l coating materials L-leucine (L; Alfa Aesar), L-valine (V; Alfa Aesar) or L-phenylalanine (P; Alfa Aesar) of 10 g/l in deionized water to form precursor solutions used in the aerosol process. Microparticles were prepared by generating solute droplets with an ultrasonic nebulizer (RBI Pyrosol 7901) which were transferred with nitrogen gas (10 l/min) to an aerosol reactor [4-6] set to 180 °C (± 2°C). At the reactor downstream, the aerosol was rapidly cooled with a large volume of nitrogen gas to initiate nucleation and deposition of amino acid vapor on the surface of drug particles, which were then collected using a cyclone. Table 1 summarizes the contents of materials in the fine powders.

Table 1. The contents of the fine powders.

Material	BP	S	L, V or P	F68	M
w-%	2.5	2.5	31.3	1.2	62.5

The size distributions were monitored using an electrical low-pressure impactor (ELPI, Dekati Ltd.). The particles were imaged with scanning electron microscopy (SEM, Zeiss Sigma VP). The aerosolization performance of the carrier-free fine powder formulations (without coarse carrier particles) was studied with an inhalation simulator [7]. Two inhalers, a multi-dose reservoir-type Easyhaler® inhaler and single-dose capsule Twister™ inhaler, were used. The Easyhaler reservoir was filled with ~ 1 g of powder, and Twister with capsules (Vcaps size 3, Capsugel) filled with 5.0 mg/capsule. Ten emissions were measured gravimetrically after each inhalation. Pressure drops over the inhalers were adjusted to 2 and 4 kPa corresponding to the inspiration flow rates of 40 l/min and 55 l/min for Easyhaler and 43 L/min and 55 L/min for Twister. The inhalation profiles were fast, that is, the maximum pressure drops (acceleration 16-18 kPa s^{-1}) and flow rates (acceleration 132.3 L $min^{-1}s^{-1}$) were achieved in two seconds maintaining these sets for 9 s and then stopped. Fine particle fractions, d ≤ 11.0 µm and d ≤ 5.1 µm, were measured gravimetrically on greased aluminium collection stages of a Berner-type low-pressure impactor, BLPI [8]. Fine particle fractions were expressed with reference to the emitted dose (ED). Micronized BP (BP_{micr}, d < 5 µm, a gift from Orion Pharma) fine powder was used as a reference.

Results and discussion
We have earlier observed that mannitol crystallizes upon particle drying [8]. Leucine and valine also crystallize whereas phenylalanine remains amorphous [9]. All amino acids are surface-active in water and thus accumulate at the gas-water

interface of the drying droplets. In the aerosol process, the amino acids were partially sublimed [9], which resulted in dry particles with differently assembled amino acid layers. The inner 'encapsulating' layer was formed by molecular diffusion within droplets, and the outer 'coating' layer was formed via vapor deposition of amino acid. The structural intactness of the particles (geometric number mean diameter (GNMD) 1.7-2.1 µm, geometric standard deviation (GSD) 1.6-1.9) denotes to physical stability without sintering with adjacent particles. Leucine hardened the particle surface (BP-S-L) preventing particle coagulation, see Fig. 1. Valine coated particles BP-S-V were sintered to some extent but phenylalanine coated particles BP-S-P were strongly sintered. Fig 1 also shows the particle assembly in a single particle wherein the nanocrystals of beclomethasone dipropionate and amorphous salbutamol sulphate are embedded in mannitol matrix that is encapsulated and coated with amino acid.

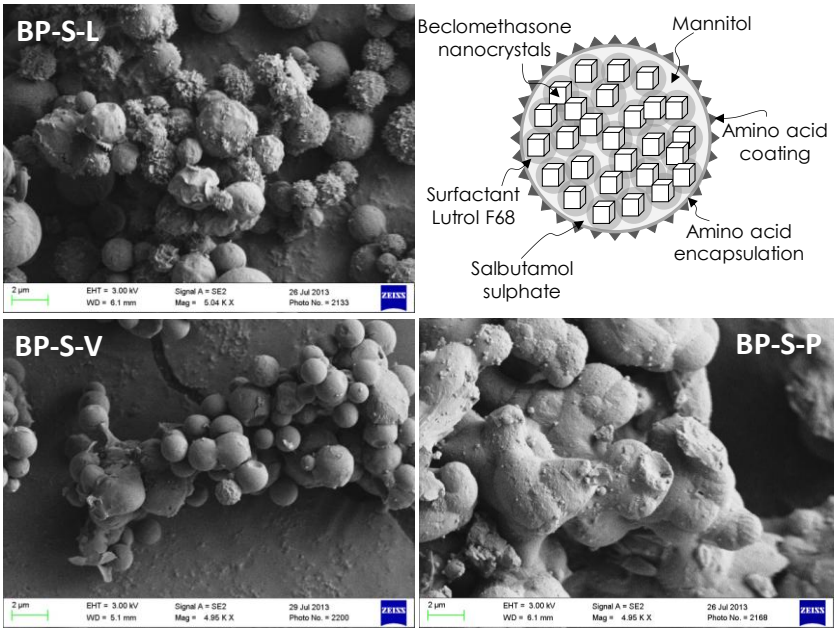

Figure 1. SEM images of the combination drug powders with different amino acid coating. Particle assembly of intact particle is described in the inset. Scale bar is 2 µm.

Powder delivery properties were studied without any additional course carrier particles commonly used in DPIs. Tables 2 and 3 show the aerosolization results conducted with Easyhaler and Twister, respectively.

Table 2. Aerosolization results of the carrier-free fine powders from Easyhaler at two pressures and inhalation flows, 2 kPa = 40 l/min and 4 kPa = 55 l/min (n = 10; SD = 5 %). ED = average emitted dose; CV_{ED} = coefficient variation of emitted dose; FPF = fine particle fraction

	ED (mg/dose)		CV_{ED}		FPF (%, d<5µm)		FPF (%, d<11µm)	
Sample	2 kPa	4 kPa	2 kPa	4 kPa	2 kPa	4 kPa	2 kPa	4 kPa
BP-S-L	3.5	3.6	0.13	0.11	47	49	62	62
BP-S-V	1.1	1.1	0.07	0.17	15	23	25	39
BP-S-P	3.1	3.3	0.13	0.12	4.3	4.0	11	10
BP_{micr}	1.0	0.8	0.30	0.33	5.2	5.7	7.0	7.6

Table 3. Aerosolization results of the carrier-free fine powders from Twister at two pressures and inhalation flows, 2 kPa = 43 l/min and 4 kPa = 55 l/min (n = 10; SD = 5 %). ED = average emitted dose; ED_{eff} = efficiency of emission; CV_{ED} = coefficient variation of emitted dose; FPF = fine particle fraction

	ED (mg/dose)		ED_{eff} (%)		CV_{ED}		FPF (%, d<5µm)		FPF (%, d<11µm)	
Sample	2 kPa	4 kPa	2 kPa	4 kPa	2 kPa	4 kPa	2 kPa	4 kPa	2 kPa	4 kPa
BP-S-L	3.6	4.2	71.8	83.8	0.11	0.06	40	40	51	47
BP-S-V	4.1	4.0	82.2	80.0	0.12	0.04	18	26	30	40
BP-S-P	4.0	4.4	80.6	87.6	0.09	0.05	3.5	3.4	8.9	8.6
BP_{micr}	3.1	3.4	61.8	67.8	0.18	0.15	27	51	31	55

From the Easyhaler, the BP-S-L and BP-S-P powders had high ED but due to different reasons. The BP-S-L particles are separated and easy to detach from each other and inhaler surface due to a rough, hard crystalline surface whereas the BP-

S-P particles emitted due to aggregate bulkiness similar to course carrier particles. As a consequence, the FPF of BP-S-P was 5-11 times lower than that of the BP-S-L. The BP-S-V and BP_{micr} showed poor emissions and poor to moderate FPFs, most probably due to the cohesive nature of powder. Dose repeatability, which is described as CV_{ED} were relatively low with amino acid containing particles but increased 2-3 fold in case of BP_{micr}. Also, EDs and FPFs between pressure drops were similar.

From the Twister, the EDs of all the powder samples, even of BP_{micr}, were relatively good between 3.1-4.4 mg/dose but the CV_{ED} values were lower (i.e. better dose repeatability than those with Easyhaler as the pressure drop and flow rate increased). The efficiency of emission (ED_{eff}) from capsules was 72-88 % with the amino acid containing powders. BP_{micr} showed not only lower efficiency (ED_{eff} = 62-68 %) but also poorer dose repeatability than the amino acid containing particles. FPFs between BP-S-L, BP-S-V and BP-S-P were in the same order as with Easyhaler but interestingly BP_{micr}, which was strongly cohesive, showed relatively high FPF, similar to that of BP-S-L. However, the BP_{micr} powder showed strong dependency on the inhalation flow rate and pressure drop whereas the FPF of BP-S-L powder was independent in this respect. It is noted that the device interior of Twister appeared to be much more 'powdered' after the BP_{micr} than BP-S-L, BP-S-V and BP-S-P. Mass medium aerodynamic diameters and geometric standard deviations of the aerosolized particles could not be accurately determined due to the lack of collection of particles larger than 11 µm.

Conclusions

Amino acids showed very different encapsulating and coating characteristics in terms of particle integrity and aerosolization properties. All used amino acids were surface-active in water and most likely accumulated at the droplet surface and subsequently particle surface. However, the particle integrity seemed to correlate with how amino acid molecules are assembled at the surface. The most stable particles were obtained with leucine, which was fully crystalline whereas amorphous phenylalanine was unable to harden the surface [9]. Moreover, the leucine rough coating performed the best aerosolization properties in terms of emission and fine particle deposition and also independency of applied pressure drop inhalation flow rate.

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