Effect of Interactive Ternary Mixtures on Dispersion Characteristics of Ipratropium Bromide in Dry Powder Inhaler Formulations

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ABSTRACT

The purpose of this investigation was to evaluate the effect of mixing order and the influence of adding fines on in vitro performance of ipratropium bromide (ITB) dry powder inhaler formulations. Coarse lactose (CL) in varying mass ratio with or without addition of micronized lactose (ML) and ITB in different mixing sequences was used to formulate ternary mixtures. A binary mixture composed of CL and ITP served as control. The in vitro deposition of ITB from these formulations was measured using an Andersen cascade impactor (aerosolization at 39 L/min) employing a HandiHaler as the delivery device. It was observed that mixing order has a significant effect ($P < .05$) on in vitro deposition of ITB. Formulations with preblending of CL and ITB produced similar deposition profiles as the control, regardless of the added ML. In contrast, formulations without preblending resulted in significantly higher fine particle dose (FPD) as compared with the control. In addition, an increased quantity of ML generally resulted in an increase in drug deposition. The results show that the effect of ML on dispersion of ITB is highly dependent upon the mixing order. The evaluation of atomic force measurement (AFM) to forecast drug detachment and predict the aerodynamic characteristics resulted in similar attraction forces for the different pairs lactose/lactose (42.66 ± 25.01 nN) and lactose/ITB (46.77 ± 17.04 nN).

KEYWORDS: Ternary interactive mixture, mixing order, atomic force microscopy, interparticle forces, dry powder inhaler, lactose.

INTRODUCTION

Dry powder inhaler (DPI) formulations require respirable drug particles with an aerodynamic diameter of 1 to 5 μm and good flow properties.[1] To fulfill these requirements, interactive mixtures consisting of micronized drug particles adhered to the surface of coarse carrier particles have been employed to reduce the interparticle forces between drug particle and coarse carrier.[2] In this case, redispersion of the drug particle from interactive mixtures and retention in the inhaler device as well as deposition of the coarse lactose in the oropharyngeal region is taking place after inhalation. Physicochemical particle characteristics, eg, size, shape, surface morphology, contact area, and surface roughness, may strongly influence particle interactions and drug dispersion.[3-6] The addition of ternary components (fine carrier particles) to interactive powder mixtures, eg, micronized lactose or magnesium stearate, has been suggested to improve the efficiency of lung deposition of aerosolized drug.[7] This has been shown for example for salbutamol sulfate[1,4,7] and beclomethasone dipropionate[8]; however, the mechanism of effects between the ternary components and drug dispersion has not been fully elucidated.[3,9] Saturation of active sites on the carrier particle has been proposed as one mechanism to increase drug deposition, where the adhesion of the ternary component on the active (high-adhesion) sites may leave the passive (low-adhesion) site for drug adhesion.[1,10] Therefore, interparticle forces between drug and carrier may be reduced[11] and an increase in drug detachment has been suggested.

A study by Zeng et al[7] reported on the effect of the addition of micronized lactose and the mixing order on the FPD of salbutamol sulfate. They employed different flow rates and devices for their investigations. They concluded, that the mixing order may be more important in determining pulmonary drug delivery when formulations are aerosolized at relatively low flow rates via inhalers of low air resistance. They also concluded that the control of mixing order may be a particularly useful strategy in the preparation of powder formulations intended to be aerosolized at low flow rates. This conclusion is challenged in the present work by employing a device with a high air resistance.

A variety of indirect techniques has been developed to measure adhesion forces for powder materials: vibration, centrifuge techniques, and impact separation.[3,12,13] With the advent of atomic force microscopy (AFM) and the development of colloid probe techniques, quantification of the interaction force between an individual drug particle and a

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substrate surface can be determined as a function of sample displacement.[13-15] For that purpose, the deflection of a spring-like probe is recorded as the substrate is brought in and out of contact with the particle. Besides adhesion measurements, an in vitro test to quantify the respirable amount of drug emitted from an inhaler is a necessary requirement to elucidate the performance of ternary mixtures.[16,17]

The objective of this investigation was to examine the effect of a ternary mixture on dispersion characteristics of ipratropium bromide (ITB) by varying the ratios of ternary component and secondly by varying the mixing sequence of all 3 components. Formulations were aerosolized at a low flow rate via an inhaler of high air resistance.

The feasibility of atomic force microscopy measurements for quantifying the interparticle forces between the different components and, as a result, to elucidate the mechanism of a ternary mixture was also studied.

Materials and Methods

Materials

Micronized ipratropium bromide (volume mean diameter [VMD] = 5 μm) was a gift sample from Boehringer Ingelheim Pharma GmbH and Co KG (Ingelheim, Germany). Respitose 325 M (VMD = 65 μm; DMV International, Veghel, The Netherlands) was used as coarse carrier without further modification. Micronized lactose (VMD = 10 μm; ML) was produced by means of a spiral jet mill (Neo-Mikro, Typ MS20DT) from coarse lactose (feeding rate 130 g/min, grinding pressure 6.0 bar). The HandiHaler, a pulmonary delivery device that is used worldwide, and polyethylene capsules (size 3) were obtained from RPC Formatec GmbH and Co KG (Mellerichstadt, Germany).

Sodium 1-heptanesulphonate monohydrate and orthophosphoric acid were purchased from Fluka (Deisenhofen, Germany). Absolute ethanol (96%) and hydrochloric acid (1 mol/L) were obtained from Riedel-de-Haen (Seelze, Germany). Acetonitrile (HPLC grade) was supplied by Merck (Darmstadt, Germany) and deionized water was generated by a Milli-Q water filtration system (Millipore GmbH, Eschborn, Germany). Anhydrous glycerol was obtained from Grüssing GmbH (Filsum, Germany). Brij35 (polyoxyethylene lauryl ether) was from Serva (Heidelberg, Germany). All chemicals and reagents used were of analytical grade or pharmaceutical grade.

Preparations of interactive mixtures

The manufacture of the binary and ternary interactive mixtures was done according to a fixed protocol ("sandwich method"). Four test formulations were prepared using different sequences of powder component addition (Table 1).

Table 1. Mixing Sequences and Detailed Composition (% wt/wt) for the Preparations of Ternary Ordered Mixtures Composed of ITB, CL, and ML*

	Mixing Sequences		
Formulation	Premixture	Final component	Detailed Composition (ITB: CL: ML)
Control	ITB + CL	—	0.7: 99.3
A	ITB + CL + ML	—	0.7: 96.8: 2.5
B	ITB + CL + ML	—	0.7: 94.3: 5.0
A´	ITB + CL	ML	0.7: 96.8: 2.5
B´	ITB + CL	ML	0.7: 94.3: 5.0

*ITB indicates ipratropium bromide; CL, coarse lactose; ML, micronized lactose; —, no final component added.

Each test formulation contained coarse lactose (CL) in varying mass ratios with addition of micronized lactose (ML) plus ITB (0.7% wt/wt).

In a first series, CL, ML, and ITB were sieved (mesh size: 0.315 mm) in layers followed by mixing in a T2 C Turbula mixer (Willi Bachofen AG, Basel, Switzerland) for 30 minutes at 900 rev/min.

Mixing in layers was performed by sieving a first layer of CL followed by a layer of ML and finally a layer of ITB. This sequence was continued until all material had been combined (30 ± 5 layers). The blend from the Turbula mixer was sieved again and mixed for an additional 30 minutes. This method of mixing ensured that drug agglomerates were destroyed and blend uniformity was optimized.

In a second series, CL and ITB were first sieved separately (15 ± 3 layers) and mixed for 30 minutes (= premixture) using a Turbula mixer before addition of the ML (15 ± 3 layers) followed by blending for an additional 30 minutes. The mixture was sieved and blended for another 30 minutes. A formulation composed of only CL and ITB was prepared as control formulation in the same way.

All blends containing ITB were manually filled in polyethylene capsules (size 3) via a dosing tube. Each capsule contained 5.5 ± 0.5 mg of the powder.

Characterization of Dry Powder Inhaler Formulations

Determination of homogeneity of the mixtures

Ten samples (16.5 mg accurately weighed) were taken at random from the blend (total blended mass 250 g) via a dosing tube. Powder blends were accepted as homogeneous and used for further testing when relative standard deviation (RSD) was less than 6.0%.

Each sample was assayed by validated high-performance liquid chromatography (HPLC)-UV spectrophotometry at a

wavelength of 210 nm for ITB. The HPLC system consisted of a Waters 2690 separation module (Waters GmbH, Eschborn, Germany) coupled with a Waters 2996 photodiode array detector. Sample analysis was performed by a reverse-phase HPLC assay, using a 125 × 4 mm, 5-μm LiChrospher 60RP Select B column (Merck). The mobile phase was 76% acetonitrile and 24% buffer solution (0.25% wt/vol sodium 1-heptanesulphonate monohydrate and water, orthophosporic acid; pH 3.2) at a flow rate of 2.0 mL/min. Quantification of ITB concentrations was achieved by the external standard technique (2.1 μg/mL). Limit of Quantification was 1 ng/100 μL and Limit of Detection was 0.5 ng/mL.

Deposition method

Aerodynamic particle size distribution of ITB was determined using an Andersen 8-stage cascade impactor (ACI) (Thermo Anderson, Smyrna, GA) after aerosolization of 6 capsules (= one collective) using a HandiHaler as delivery device at a flow rate of 39 L/min for 6.15 seconds (4-L volumes) for each capsule.

With respect to the flow control equipment, the operation conditions for the ACI were different from 28.3 L/min (European Pharmacopoeia [PhEur] and US Pharmacopeia [USP]) and were therefore validated.[18] According to the PhEur, alternative flow rates may be employed, which requires recalculation of the cutoff limits. The cut off of the ACI under these conditions is calculated by the following equation:

$$\text{Cut off}_{39\ L/\text{min}} = \text{cut off}_{28.3\ L/\text{min}} \sqrt{28.3\ L/39\ L} \quad (1)$$

The ACI consists of a sample induction port (SIP), a USP high top (connection between the sample induction port and preseparator), a preseparator, and 8 impaction stages with stainless steel collection plates, which were coated with 50 μL of a coating reagent (Brij 35/ethanol mixture in glycerol). A binderless borosilicate glass-fiber filter (diameter 76 mm, pore size 1 μm, retention capacity ≥ 99.98%) was placed after the bottom impaction stages. With the mouthpiece of the inhaler coupled on axis with the entry to the sample induction port, each capsule was actuated 1 time. After delivery of the powder of 6 capsules, the impactor was dissembled and the amount of ITB was recovered quantitatively from the capsule shells, preseparator, sample induction port, collection plates, and filter by washing 3 times with 0.01 mol/L aqueous hydrochloric acid. Samples were then assayed by HPLC-UV spectrophotometry as described above.

Each capsule contains a nominal dose of 5.5 ± 0.5 mg powder, equivalent to 40 ± 3.6 μg ITB. At least 4 collectives were tested for each formulation. A variety of parameters were employed to characterize the deposition profiles of ITB in ACI. The recovered dose (RD) was taken as the total quantity of drug recovered per capsule after each actuation (cumulative mass of ITB from SIP, preseparator, plate 0 to filter, and remains in capsule), while the emitted dose (ED) was that emitted from the inhaler device (cumulative mass of ITB from SIP, preseparator, and plate 0 to filter). The total recovery (% recovery) of the drug was calculated as the percentage of the RD to the theoretical dose of ITB. In addition to the fine particle dose (FPD), defined as particles with effective cutoff diameter (ECD) < 4.9 μm (grouping 2 and 3), the different stages were divided into 3 groupings. Grouping 1 was the amount of drug collected on plates 0 to 1 (coarse: 4.9 μm to 8.5 μm), grouping 2 (fine: 1.8 μm to 4.9 μm) was denoted as the quantity (μg) of the particles on plates 2 to 4, and grouping 3 (very fine: < 0.3 μm to 1.8 μm) was the quantity of drug collected on the plates 5 to filter.

Adhesion force measurements

AFM measurements were performed using a MultiMode AFM controlled with Nanoscope IIIa and Nanoscope Extender Electronics (AFM; Digital Instruments/Veeco Technology, Santa Barbara, CA). All images were acquired in normal air using Tapping Mode with "Vertical J" scanner (Digital Instruments, Veeco Metrology, Santa Barbara, CA) (150 μm). For particle adhesion measurements, a selected single 5-μm diameter crystal of lactose respectively ITB was glued using epoxy glue at the free end of the V-shaped silicon cantilever (spring constant: 5.29 to 16.88 N/m). As sample, a small amount of lactose was dropped onto a cleaned 15-mm stainless steal disc coated with double-sided adhesive.

The deflection of the modified cantilever due to the force produced by the sample was recorded using the software analysis program NanoScope operating software version 4.42r4 running under Windows NT (Digital Instruments/Veeco Metrology). The contact point is defined as the breaking point where the slope of the force versus separation curve changes abruptly (pull of distance). The adhesion forces were determined using Hook's law, where the vertical cantilever displacement was multiplied with the cantilever spring constant. The adhesion force distribution of each sample was obtained from adhesion force measurements realized at 10 individual sample areas. All measurements were performed at 55% relative humidity in normal air at room temperature.

Scanning electron microscopy

Surface morphology of the mixtures was examined visually by scanning electron microscopy (SEM) on a Leo supra 55VP (Zeiss NTS GmbH, Jena, Germany) with quadral backscattering detector (QBSD) and variable pressure secondary electron detector (VPSED). Samples were glued onto aluminum

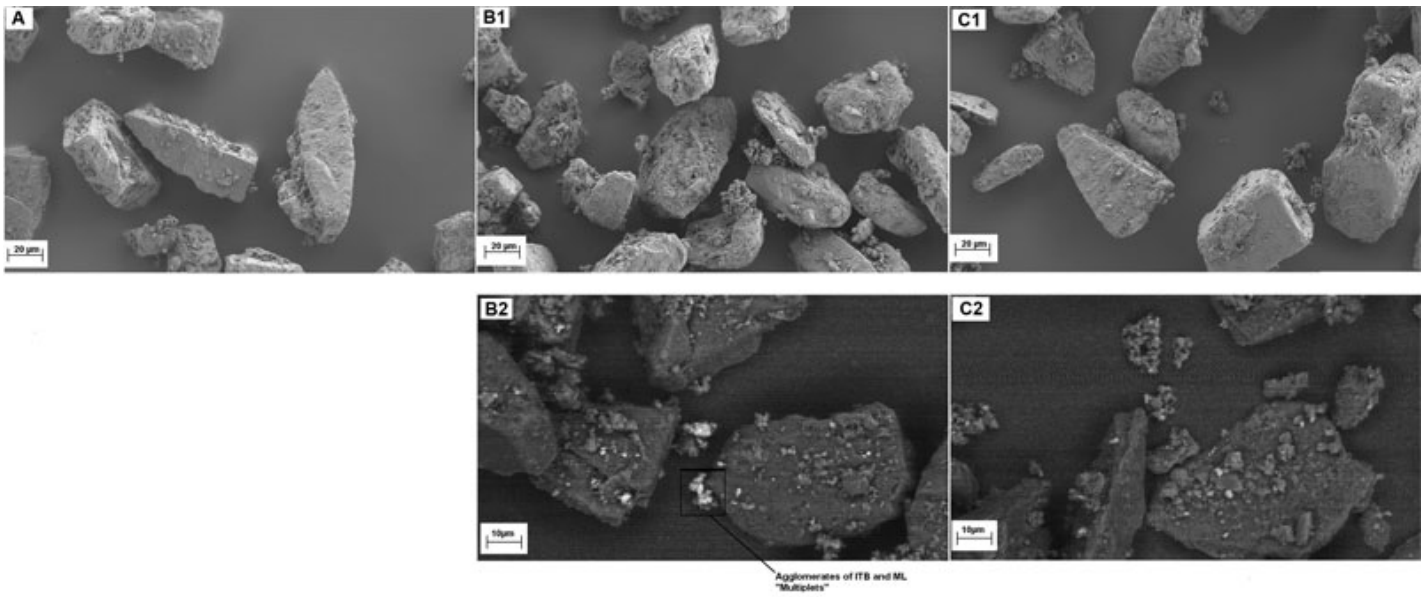

Figure 1. Scanning electron micrographs with secondary electron detector of (a) coarse lactose (CL); (b1) formulation A and (c1) formulation A′ (EHT = 1.00 KV; High Vacuum; Signal A = SE2) and with quadral backscattering detector of (b2) formulation A and (c2) formulation A′(EHT = 15.00 KV; variable pressure; Signal A = QBSD); white spots = ITB.

sample stubs and viewed using variable pressure or high vacuum at 1-kV current.

Statistical analysis

For each of the parameters FPD, grouping 1, grouping 2, and grouping 3, an analysis of variance (ANOVA) with the fixed factors *micronized lactose*, *mixing order*, and the corresponding *interaction term* was conducted to examine the average differences between the deposition data of the different factor levels. Since the overall considerations for micronized lactose and mixing order show significant differences, pairwise comparisons with the common error term of the ANOVA were applied to investigate the average differences between the individual formulations used. All statistical tests were performed with the 2-sided significance level of .05, using the procedure MIXED of the SAS system (SAS Institute Inc, Cary, NC, version 8.2).[19,20]

Results and Discussion

Characterization of the different blends

All mixtures were homogeneous with a coefficient of variation in ITB content of less than 3.3% (n = 10). The existence of interactive mixtures was confirmed by SEM where drug and ternary component association with the coarse carrier surface was observed (Figure 1). Particle adhesion on the carrier was nonuniform and characterized by aggregation of drug particles. No obvious difference in content uniformity and adhesion morphology between the formulations with different mixing orders was illustrated.

Aerodynamic particle size distribution of ipratropium bromide

No significant differences were observed in the ED and the RD of ITB from all formulations, suggesting that mixing order and added ML did not have a significant effect on the total amount of ITB delivered after aerosolization (Table 2). Nevertheless, the various formulations produced significantly different FPD and groupings of ITB ($P < .05$). As described in Table 3, the formulations without a premixture (A and B) produced significantly higher FPD of ITB as compared with the control (control < A < B; $P = .0008$ /.0001), whereas the formulations with premixture (A′ and B′) produced significantly lower FPD as compared with the control (control > A′ ~ B′).

The formulations containing the same amount of ML yet different mixing sequences for their preparation (A - A′ and B - B′), yielded the lowest FPD for those formulations with premixture of CL and ITB (Table 2). For example, formulation A resulted in an FPD of 13.7 ± 0.6 µg, which was nearly twice the amount observed with formulation A′. The FPD of the control formulation was equivalent to the formulations with premixture ($P = .1429$) but lower than those without premixture ($P < .008$). Therefore, the adding sequence of ML was statistically significant (Table 4) with respect to FPD when compared with a binary mixture ($P < .0001$). The results of the formulations A and B were further in agreement with those reported elsewhere, where the adding of fines resulted in increased FPD.[17] Formulation A (fines: 2.5% wt/wt) produced significantly higher FPD ($P = .0008$) than the binary control mixture and significantly lower FPD ($P = .0066$) than formulation B (fines: 5.0% wt/wt).

Table 2. Deposition of ITB From Different Formulations in Polyethylene Capsules at 39 L/min via HandiHaler (means ± SD; n = 4)*

Formulation	Grouping 1, μg	Grouping 2, μg	Grouping 3, μg	FPD, μg	ED, μg	RD, μg	Recovery, %
Control	1.3 ± 0.1	9.2 ± 0.7	1.1 ± 0.3	10.3 ± 0.9	31.2 ± 0.6	34.5 ± 0.7	86.3 ± 1.8
A	1.9 ± 0.2	12.0 ± 0.7	1.6 ± 0.1	13.7 ± 0.6	30.9 ± 0.6	34.2 ± 0.3	85.4 ± 0.7
B	2.5 ± 0.2	14.8 ± 0.4	1.5 ± 0.2	16.3 ± 0.6	32.4 ± 1.3	35.6 ± 1.1	89.0 ± 2.8
A′	2.4 ± 0.4	7.1 ± 0.9	0.4 ± 0.1	7.5 ± 0.9	32.6 ± 2.1	34.9 ± 1.6	87.2 ± 4.0
B′	2.3 ± 0.1	8.4 ± 1.2	0.5 ± 0.2	8.9 ± 1.3	32.4 ± 1.0	34.6 ± 1.4	86.5 ± 3.6

*FPD indicates fine particle dose; ED, emitted dose; RD, recovered dose.

For a closer examination of the effects of mixing order and added ML, the groupings were analyzed in more detail (Tables 3 and 4).

Grouping 1 (plates 0 to 1)

All ternary mixtures produced significantly higher grouping 1 of ITB as the control ($P < .0119$). Unaffected of the different quantities (2.5% or 5%), the addition of ML was shown to improve the deposition of ITB, while the mixing sequence has a minor effect on the ITB particles on plate 0 to 1 (Figure 2). This observation was confirmed by SEM photomicrographs of ITB formulations (Figure 1). ML adhered either to the coarse carrier particle ("coating effect") or formed small aggregates ("multiplets") with ITB. This resulted in efficient detachment of ITB from the carrier as observed with binary mixtures.[12,17,21]

Grouping 2 (plates 2 to 4)

As illustrated in Figure 2, the grouping 2 of formulations without premixture (A and B) were in agreement with those reported for FPD. A statistically significant increase ($P = .0014$) in the quantity of particles collected on plates 2 to 4 was observed when increasing the amount of ML from 2.5% to 5% (wt/wt). Furthermore, both ternary mixtures without premixture yielded higher grouping 2 as the binary control mixture. Even the ternary formulations with premixture (A′ and B′) were in agreement with the previously reported FPD, where subsequent addition of ML to the premixture of CL and ITB did not improve the dispersion of ITB (Table 3 and Figure 2). For example, formulation A′ yielded statistically significant lower grouping 2 than the control formulation ($P = .0098$), while no significant difference was observed between formulations A′ and B′ ($P = .869$).

These data revealed an effect of adding sequence of ML. In formulations without premixture high-energy adhesion sites (HA) of the carrier may bind strongly to ML and low-energy adhesion sides (LA) may allow the formation of more reversible bonds with ITB. This action results in efficient detachment if drug from the carrier as observed elsewhere.[4,17] This phenomenon is termed "coating effect," because the carrier particles are coated with mono- and multilayers of ML. A higher quantity of ML increases this effect and the adhesion force between drug and carrier decreases because only LA sites remain. In case of a premixture of CL and ITB, the HA sites are initially saturated by drug particles and no coating effect of the ML added subsequently is observed.

Grouping 3 (plates 5 to filter)

As illustrated in Figure 2 only the mixing sequence of ML with carrier resulted in significantly different results (without

Table 3. ANOVA Results—Effect of Micronized Lactose and Mixing Order ($\alpha = 0.05$)*

		Grouping 1 (plate 0 – 1)		Grouping 2 (plate 2 – 4)		Grouping 3 (plate 5 – filter)		FPD (plate 2 – filter)	
Formulation 1	Formulation 2	Difference, μg/capsule	*P* value	Difference, μg/capsule	*P* value	Difference, μg/capsule	*P* value	Difference, μg/capsule	*P* value
Control	A	–0.62	.0004	–2.83	.0011	–0.54	.0010	–3.38	.0008
Control	A′	–1.11	.0001	2.13	.0098	0.65	.0002	2.78	.0040
Control	B	–1.14	.0001	–5.58	.0001	–0.39	.0119	–5.97	.0001
Control	B′	–1.02	.0001	0.78	.3072	0.53	.0013	1.31	.1429
A	A′	–0.49	.0032	4.96	.0001	1.20	.0001	6.16	.0001
A	B	–0.52	.0019	–2.75	.0014	0.15	.3000	–2.60	.0066
A′	B′	0.09	.5272	–1.35	.0869	–0.12	.3918	–1.47	.1029
B	B′	0.13	.3933	6.37	.0001	0.92	.0001	7.29	.0001

*Significance level $\alpha = 0.05$. ANOVA indicates analysis of variance; FPD, fine particle dose.

Table 4. ANOVA Results—Pairwise Comparison by 2-Sided *t* Tests ($\alpha = 0.05$)*

	Grouping 1		Grouping 2		Grouping 3		FPD	
Main effect	F value	*P* value	F value	*P* value	F value	*P* value	F value	*P* value
% Micronized lactose	7.57	.0119	23.78	.0001	0.05	.8192	18.28	.0003
Mixing order	4.85	.0186	81.20	.0001	65.79	.0001	83.86	.0001

*ANOVA indicates analysis of variance; FPD, fine particle dose.

premixture < control formulation < with premixture), whereas the quantity of added ML did not have a significant effect (Table 3 and 4).

These findings were elucidated by the coating effect of ML. In case of the formulations with premixture (A′ and B′) the smallest drug particles prefer occupation of the HA sites and are not released from the lactose carrier particles after aerosolization. Within formulations without premixture, ML occupies HA sites leaving LA sites for attachment for ITB and thus resulting in higher quantities of ITB on the lower stages of the ACI.

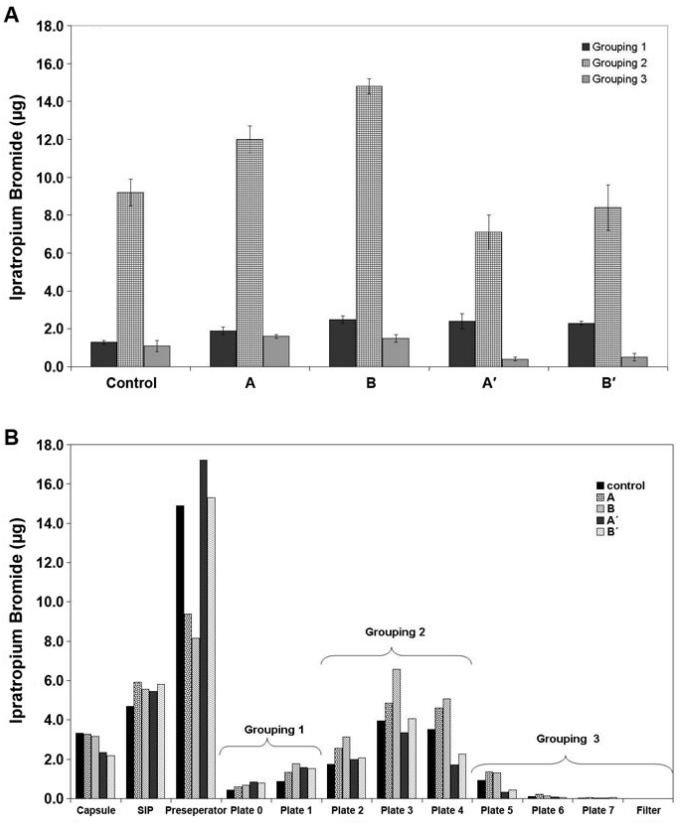

Figure 2. Deposition profile of ITB as measured by an ACI after aerosolization at 39 L/min via a HandiHaler. (A) Groupings 1–3 of the different formulations (error bars denote SD; n = 4 collectives). (B) Detailed deposition pattern of ITB blends in ACI (n = 4 collectives).

Deposition profiles of ITB in the preseparator

The effect of mixing sequence and concentration of ML on deposition profile of ITB in the preseparator was evaluated and illustrated in Figure 2. Formulations prepared by initially blending ITB and CL before mixing with ML (A′ and B′) resulted in deposition profiles similar to the binary control mixture. Whereas in formulations without premixture (A and B), the addition of ML was generally more effective in reducing the content of ITB in preseparator.

These findings confirmed the observed effect of the mixing sequence. Coating of the coarse carrier with ML resulted in a decrease of drug deposition in the preseparator. Due to the "coating effect" (mono- and multilayers) of ML, the drug particles preferred occupying LA sites and leaving the carrier surface after aerosolization. Without the coating effect (formulations with premixture of ITB and CL), the drug particles bind strongly to HA sites and are not released from carrier after aerosolization. Consequently, an increase in drug deposition in the preseparator corresponds to a decrease in FPD.

Comparison of adhesion forces

As illustrated in Figure 3 electrostatic forces between lactose monohydrate (carrier surface) and ITB probes (drug 1–5) or ML probes (lactose 1–5) were not observed. Merely

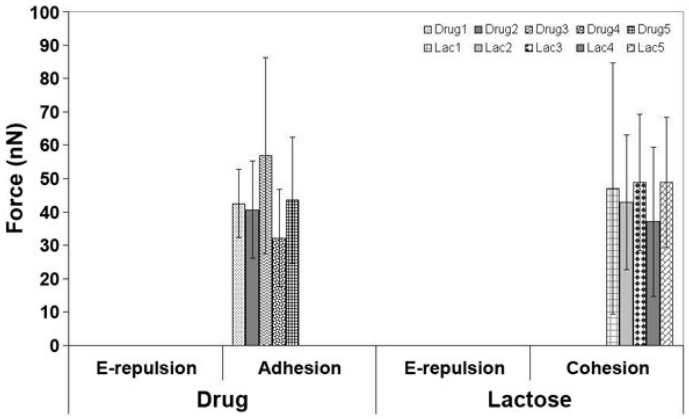

Figure 3. Comparison of adhesion forces between lactose surface and ITB (drug)/ML (lactose) crystals fixed on the AFM tip (mean value ± SD; n = 240 – 340 force scans from 10 different areas).

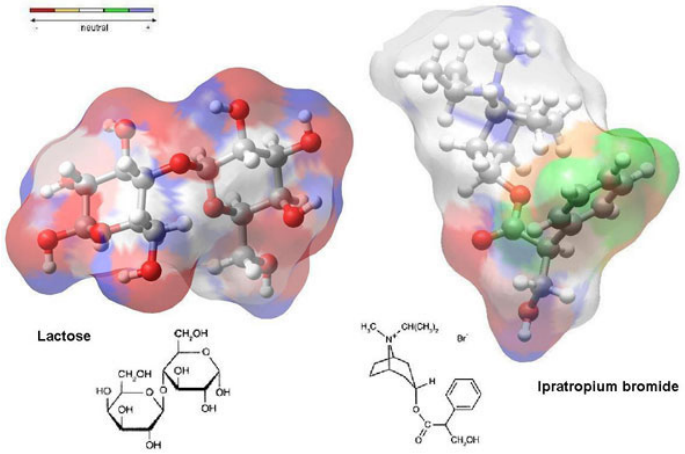

Figure 4. Partial charge of ITB and lactose (3D-model; Maestro Version 7.1 and Macro Model Version 9.0; Schroedinger, Portland OR).

small adhesive forces were detected between lactose (target) and ITB (probe) and lactose (target) and ML (probe). For example, between lactose and ITB probes, a mean adhesive force of 46.77 ± 17.04 nN (n = 240 force scans from 10 separate areas) was measured. Mean interaction forces of 42.66 ± 25.01 nN (n = 340 force scans from 10 separate areas) were observed between lactose probe and carrier lactose material such that significant differences in the magnitude of the interfacial forces between the 2 probe/target pairs were not observed ($F_{ML} \sim F_{ITB}$).

These observations were elucidated on the basis of surface charges (Figure 4). For example, a negative surface charge is predominant in the lactose molecules and causes repulsion between particles of the same material. Therefore, no long-ranging electrostatic forces yet only short-ranging forces (adhesion forces; hydrogen bonds) are detected. Even in the case of interparticle forces between ITB and lactose, only short-ranging forces were detected. This is caused by the mainly neutral surface charge of ITB and the negative surface charges of lactose.

In summary, the magnitude of interparticle forces for all components is in a similar range and cannot explain the observed aerodynamic characteristics of the drug. This latter aspect requires further testing.

Conclusion

The mixing sequence of the different components of the ternary mixture has a significant effect on the dispersion of ITB, even at low aerosolization flow rates via a high air resistance device. Adding ML to the formulation ("coating effect") appears to reduce interaction between drug and carrier by occupying possible binding sides in the coarse carrier. This study demonstrated further the feasibility of atomic force measurements for quantifying the interparticle forces between the different components and consequently aids in providing a mechanistic explanation for the findings with respect to ternary mixtures.

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