



Impact of capsule type on aerodynamic performance of inhalation products: A case study using a formoterol-lactose binary or ternary blend

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ABSTRACT

The aerodynamic performance of a dry powder for inhalation depends on the formulation and the dry powder inhaler (DPI). In the case of capsule-based DPIs, the capsule also plays a role in the powder aerosolisation and the dispersion of the micronized drug during the inhalation. This study evaluated the impact of gelatine capsules (Quali-G[™] and Hard Gelatine Capsules for DPIs), cold-gelled hypromellose (HPMC) capsules (Quali-V^{-I} and Vcaps[®]) and thermal-gelled HPMC capsules (Vcaps[®]Plus) from Qualicaps[®] and Capsugel[®] respectively, on the delivered dose (DD), fine particle dose (FPD), and capsule retention for formoterol-lactose binary and ternary blends. This study used a low resistance Axahaler[®] DPI based on the RS01 design (Plastiapipe, Italy). Similar trends were observed with the different capsule types that packaged both dry powder formulations. The highest DD and FPD and the lowest formoterol capsule retention were observed with cold-gelled HPMC capsules such as Quali-V^{-I} and Vcaps[®], without significant differences between these capsules ($p > 0.05$, one-way ANOVA with Newman-Keuls post-hoc test) for both dry powders. Therefore, the capsule composition and manufacturing process have an influence on aerodynamic performance. In addition, the ternary blend showed higher DDs and FPDs but also higher capsule retention in comparison to the binary blend.

1. Introduction

Dry powder inhalers (DPIs) are the preferred device for the treatment of an increasingly diverse range of lung diseases such as asthma, chronic obstructive pulmonary disease, and lung infections such as cystic fibrosis or to deliver some drugs systemically such as insulin (Claus et al., 2014; Yang et al., 2014). Among DPIs, there are multi-dose and multi-unit-dose based inhalers and single-dose based inhalers (Lavorini et al., 2017; Berkenfeld et al., 2015; Faulhammer et al., 2014). The latter represent almost half of all marketed DPIs and are mainly represented by capsule-based DPIs (Lavorini et al., 2017; Berkenfeld et al., 2015; Faulhammer et al., 2014). Capsule-based DPIs have the advantages of presenting accurate and consistent drug delivery and relative ease of use for the patient (Wauthoz et al., 2017). Capsule-based DPIs present excellent feedback on drug delivery to the patient on the basis of the emptied capsule (visual), the noise of the spinning capsule (auditory), and the taste of the impacted lactose carrier (taste) during and after the inhalation procedure (Lavorini et al., 2017; Berkenfeld et al., 2015; Wauthoz et al., 2017). However, the presence of the capsule increases the number of steps for the dose loading in comparison with multi-dose DPIs. This could complicate the inhalation

technique for the patient (Lavorini et al., 2017; Wauthoz et al., 2017). Nevertheless, the dose loading of the capsule-based inhaler is quite intuitive. Furthermore, the first DPI on the market (i.e. the Spinhaler[®]), several largely used single-dose DPIs (e.g. the Aerolizer[®], Handihaler[®], and Breezhaler[®]), and recent DPIs for the delivery of antibiotics (the Turbospin[®]) or multiple pre-metered unit doses (the Flowcaps[®]) are capsule-based (Lavorini et al., 2017; Berkenfeld et al., 2015). DPIs release the powder from the capsule using different motions (i.e. rotation, shake, vibration) and capsule opening mechanisms (Martinelli et al., 2015). The means to open the capsule are shear-force opening, cutting, or needles piercing the capsule (Lavorini et al., 2017). The latter is the most common method, with the number and location of piercing points varying according to the DPI. Some piercing systems produce one hole in the side wall of the cap and the body (Handihaler[®]), one or four holes in both the cap and the body ends (e.g. RS01 or Aerolizer[®], respectively), or two holes at the ends of the body (e.g. Turbospin[®]) (Martinelli et al., 2015; Schoubben et al., 2015). In addition, capsule-based DPIs facilitate the development of new chemical-entity based products for inhalation. This is because products are easily filled into capsules, allowing stability programmes to start earlier as soon as the prototype formulation is ready. Clinical studies then follow more

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quickly. Moreover, capsule-based DPIs are, by design, most suitable as disposable devices for specific diseases or developments (Lavorini et al., 2017; Wauthoz et al., 2011). Capsule-based DPIs have a more flexible range of resistance to the airflow from low (i.e. Aerolizer[®]) to high (i.e. Handihaler[®]) resistance in comparison to multi-dose DPIs that usually present medium or high resistance to the airflow (Dal Negro, 2015). Low resistance DPIs involve lower inspiratory efforts for the patient to generate the same pressure drop than a medium or a high resistance device.

On the market there are different hard capsule types used for inhalation. These follow the evolution of the development of the two-piece capsule in oral drug delivery (Jones et al., 2004). The first type was the gelatine capsule made by dipping lubricated stainless steel pins into a molten gelatine solution of a defined viscosity (Jones et al., 2004). The aqueous viscous solution on pins is then dried in air that has a controlled humidity and is heated to a few degrees above ambient temperature (22–28 °C) to form the film of the capsule shells. The lubricant or release aid put previously on the pins is necessary to prevent the film from shearing too strongly in the mould and to enable the film to slide easily over the surface of the metal mould (Jones et al., 2004). The material used is a specific and confidential formulation of a mixture of pharmaceutical grade materials registered in each capsule manufacturer's Drug Master File (Jones et al., 2004; Ayala et al., 2016). Gelatine is the primary material type used for capsules as it shows good film-forming properties and can dissolve readily in biological fluids at body temperature. However, gelatine is from animal sources, not chemically inert, and contains water (about 13–16%) that acts as plasticizer. These properties lead respectively to the risks of incompatibilities with some compounds and brittleness at low relative humidity or incompatibility for hydrolysable drugs (Nagata et al., 2001). These disadvantages have led to the development of hydroxypropyl methylcellulose or hypromellose (HPMC) capsules. These capsules use a gelling agent and network promoter (i.e. cold-gelled HPMC capsules) to allow the use of the same manufacturing processes as gelatine (Nagata et al., 2001). This type of capsule is composed of HPMC from vegetable sources. This material avoids both the risk of transmissible bovine spongiform encephalopathy and patient acceptance issues due to vegetarian dietary restrictions; is highly chemically inert, which leads to much lower incompatibilities; and contains much less water (about 3–7%), ensuring almost no brittleness upon storage at low relative humidity (Nagata et al., 2001). Gelatine capsules become brittle when they have a water content below 10%, while HPMC capsules show a highly-reduced brittleness even at 1% water content (Nagata et al., 2001). Shionogi Qualicaps Co. (Japan) developed their cold-gelled HPMC capsules with carrageenan as the gelling agent and chloride potassium as the gelling promoter, leading to the Quali-V[®] HPMC capsules (Nagata et al., 2001). Carrageenan is a linear sulphated polysaccharide extracted from red seaweeds. It forms a double helix structure connecting two molecular chains of HPMC in a three-dimensional structure. This structure results in a high gel strength and exhibits good gelling properties in combination with potassium ions at room temperature. Capsugel (Colmar, France), acquired recently by the Lonza group, developed a different HPMC gelling system using gellan gum as the gelling agent and either ethylenediamine tetra acetic acid or sodium citrate as a gelling promoter, leading to the Vcaps[®] HPMC capsules (Jones et al., 2004). Capsugel developed a thermal-gelled HPMC for oral use (Vcaps Plus[®] HPMC capsules) composed of only HPMC and water. This was done to overcome some challenges encountered with solid oral dosage forms during *in vitro* dissolution or disintegration tests in some specific conditions and with some compounds (e.g. green tea extract at pH 1.2 or in FeSSIF media), caused by using the gellan gum as a gelling agent (Glube et al., 2013). However, this new type of HPMC capsules requires a different and specific manufacturing method where the dipping pins are preheated and maintained heated to avoid the temperature dropping below the gelation temperature until the film is sufficiently dried to maintain the

capsule shape (Jones et al., 2004).

Specific capsule attributes are required for the successful administration of inhalation therapies by capsule-based DPIs. These attributes not include dissolution/disintegration tests but include, for example, moisture diffusion and permeability from and through the capsule (Barham et al., 2015), physical and mechanical performance of the capsule in a puncturing or cutting event (the hole shape, diameter, and number of holes) (Birchall et al., 2008; Coates et al., 2005), and lubricant content on the inner surface (Saim and Horhota, 2002; Saleem et al., 2015). Inhalation therapy is an interconnection between three major factors: the device, the formulation, and the patient. The capsule is an additional important factor for capsule-based DPIs, as shown by Coates et al. with the Aerolizer[®]. The capsule plays a significant role, not only in the packaging of the formulation but also in the aerosolization of the powder and the dispersion of the micronized drug after the patient has pierced the capsule and inhaled through the DPI (Martinelli et al., 2015; Coates et al., 2005). However, there are few studies evaluating the impact of the capsule type on aerodynamic performance. Therefore, the aim of this work is to evaluate rigorously and deeply the impact of different capsule types (i.e. gelatine, cold-gelled, or thermal-gelled HPMC capsules) on the aerodynamic performance of two dry powder formulations for inhalation based on lactose carrier and using formoterol as a low drug dosage form. Low resistance Axahaler[®] capsule-based DPI (SMB, Belgium) was chosen as this DPI is based on the design of the 100 L/min RS01 elaborated by Plastiap (Italy). This device is a refinement of the Aerolizer[®] for which Coates et al. have investigated the role of the capsule (size, hole number and size) on the aerodynamic performance using the computational fluid dynamics and experimental analysis (Coates et al., 2005). The main differences are the sharp bevelled piercing system leading to one hole at each end of the capsule in contrast to the four holes from conical needles for the Aerolizer[®], as photographed by Birchall et al. (Birchall et al., 2008). Moreover, RS01 DPIs were chosen as they represent the major capsule-based DPI systems (i.e. piercing) and as they are often used in research (Martinelli et al., 2015; Elkins et al., 2014; Schoubben et al., 2015) and in new or generic (Martinelli et al., 2015) products on the market (e.g. the Onbrez[®] Breezhaler or the Formagal[®] Axahaler). This work evaluated different type of capsules (i.e. gelatine, cold-gelled and thermal-gelled HPMC) mostly marketed for the inhalation field, except Quali-G[™] capsules (QG), which are marketed for oral administration. This work used QG and Quali-V[®]-I (QVI) capsules from Qualicaps[®] for gelatine and cold-gelled hypromellose (HPMC) capsules, respectively. For gelatine, cold-gelled HPMC, and thermal-gelled HPMC capsules, respectively, we evaluated also Hard Gelatine Capsules for DPIs (HGC), Vcaps[®] (VC), and Vcaps[®]Plus (VC+) capsules from Capsugel[®]. The microbiological requirements of non-sterile dosage forms are stricter for inhalation use than for non-aqueous preparations for oral use (Pharmacopeia, 2014). All capsules used in this study respected the microbiological requirements for both inhalation and oral use (Pharmacopeia, 2014).

Two dry powder blends were produced to represent typical dry powder formulations found on the market in order to evaluate the impact of the capsule type on aerodynamic performance. The blends were produced using the same micronized formoterol batch but with different formulation strategies. A binary mixture was composed of milled lactose presenting a broad particle size distribution (PSD) and a ternary mixture was composed of sieved lactose presenting a narrow PSD with the addition of 10% of fine lactose.

2. Materials and methods

2.1. Materials

Milled lactose carrier with a broad PSD characterised by a Dv50 of 46 µm and a span of 3.13 (Respitose ML001, Fig. S1 in the supplementary data), sieved lactose carrier with a narrow PSD characterised by a Dv50 of 113 µm and a span of 1.11 (Respitose SV010, Fig. S1 in the

Table 1
Main characteristics from the certificate of analysis provided by the capsules' manufacturers.

	HGC	QG	VC	QVI	VC+
Producer	Capsugel	Qualicaps	Capsugel	Qualicaps	Capsugel
Batch	53381721	R1301546	53293111	E1307099	53335011
Major material	Gelatine	Gelatine	HPMC	HPMC	HPMC
Loss on drying (%)	15.5	14.3	3.9	5.5	5.4
Lubricant content	186 ppm	N.R.	227 ppm	N.R.	213 ppm
Field application	Inhalation	N.R.	Inhalation	Inhalation	Inhalation

N.R.: not reported.

supplementary data), and fine lactose characterised by a Dv50 of 3 µm and a Dv90 of 7 µm (Lactohale LH300, Fig. S1 in the supplementary data) were kindly donated by DFE Pharma (Goch, Germany). Micronized formoterol fumarate dihydrate characterised by a Dv50 of 1.5 µm, with 99.81% < 5 µm (formoterol), was purchased from Chemo (Madrid, Spain). Size 3 capsules were kindly donated by Qualicaps® (QG and QVI for gelatine and cold-gelled HPMC capsules, respectively) and Capsugel® (HGC, VC, and VC+ for gelatine, cold-gelled, and thermal-gelled HPMC capsules, respectively). The main characteristics of each capsule type enclosed in the certificate of analysis are reported in Table 1. Low resistance Axahaler® capsule-based DPIs were kindly donated by SMB (Brussels, Belgium). Potassium phosphate and HPLC-grade acetonitrile were purchased from Merck (Darmstadt, Germany) and HPLC-grade methanol from VWR (Fontenay sous Bois, France).

2.2. Methods

2.2.1. Capsule characterization

Characterization of empty capsules was performed on capsules stored at 20 °C and 50% relative humidity (RH) in Pharma 600 chamber WeissTechnik® (Liederkerke, Belgium). This was within the range of the recommended conditions in the certificate of analysis (i.e. 15–25 °C and 35–65% RH) for Capsugel or in Qualicaps' Technical Manual (i.e. 15–30 °C and 35–55% RH).

2.2.1.1. Water content – thermogravimetric analysis (TGA). The amount of adsorbed or bound water in each capsule was assessed on the entire cap set. This was done with a platinum basket by TGA using a Q500 apparatus (TA instruments, Zellik, Belgium) and Universal Analysis 2000 version 4.4A software (TA Instruments, Zellik, Belgium). Runs in sextuplicate were set from 27 °C to 250 °C at a heating rate of 10 °C/min. The water percentage was determined by the weight loss obtained between 27 °C and 180 °C.

2.2.1.2. Hole examination after piercing and inhaling through the Axahaler DPI – scanning electron microscopy (SEM). Ten capsules of each type were successively placed in the Axahaler DPI to be pierced and then lifted up and spun using an airflow of 100 L/min. This was done to take pictures of the most representative hole type by SEM, using an SU-70 (Hitachi, Japan). Samples (cap) were put on a thin film of a two-component epoxy resin and then coated with gold. Acceleration during observation was 25 kV. A magnification of 30 × (bar scale of 1.00 mm) was applied.

2.2.1.3. Inner and outer surface evaluation – SEM and confocal microscopy. Evaluation of the inner or outer surface of the capsules (body) was achieved by SEM using an SU-70 (Hitachi, Japan) after cutting through the capsule shell. Samples were put on a thin film of a two-component epoxy resin and then coated with gold. Acceleration during observation was 25 kV. A magnification of 500 × (bar scale of 100 µm) was applied.

Evaluation of inner surface of the capsules (body) was achieved by an OPTELCIS H1200A-WID confocal microscope (Lasertec, Japan) after

having cut through the capsule shell. Samples were fixed on a glass plate by glue. A magnification of 100 × was applied and a 180 µm × 180 µm picture was taken corresponding to 1024 × 1024 data points using the LM eye software Ver4.17 (Lasertec, Japan). The data calculated were the “Sa arithmetical mean height” corresponding to the surface roughness, the “average depth of crater” corresponding to Sv (maximum pit height), the “average diameter of crater” calculated by fitting the perfect circle shape to each crater and the “total volume of craters” by using circle shape (xy coordinate data) as described above and using their z coordinate data.

2.2.2. Dry powder preparation and characterization

2.2.2.1. Dry powder formulations. The blending was performed in a 100 mL-plastic vessel filled to 40% at most of its inner volume using a laboratory-scale three-dimensional motion mixer, the Turbula 2C (Bachofen AG, Switzerland) at 46.2 rpm, as follows.

The binary mixture was made by blending coarse lactose with a broad PSD (Respitose ML001) and formoterol (0.05% w/w). First, 12 mg of formoterol was set in with 12 g of coarse lactose using the so-called sandwich method and all the powder was sieved (224 µm sieve). The mixture was then blended for 5 min. The obtained pre-mix was then set in sandwich with an additional quantity of 12 g of coarse lactose and all the powder was sieved then blended for 10 min. Then, the blend was sieved again before being blended for another 10 min.

The ternary mixture was made by blending coarse lactose with a narrow PSD (Respitose SV010), fine lactose (Lactohale 300), and formoterol (0.05% w/w). First, 12 mg of formoterol was set in with 2.4 g of coarse lactose and 2.4 g of fine lactose using the so-called sandwich method and all the powder was sieved (224 µm sieve). The mixture was then blended for 10 min. The obtained pre-mix was then sieved with 19.2 g of coarse lactose and blended for 15 min. Then, the blend was sieved again before being blended for another 10 min.

Then, the blends were stored at 20 °C and 50%RH at least 3 days before placing them into the different capsule types.

2.2.2.2. Uniformity of drug content. The test B for uniformity of content of single-dose preparations in the European Pharmacopoeia was carried out to evaluate the uniformity of drug content (Pharmacopoeia and Test, 2017). This test consists of determining the individual formoterol content of 10 dosage units (Pharmacopoeia and Test, 2017). As the capsules were manually filled, ten samples of each blend were carefully taken from different places in the mixture. For each sample, 24 ± 1 mg was weighed accurately (to 0.01 mg precision) directly in a 50-mL volumetric flask, which was then filled and sonicated for 20 min with a dilution phase consisting of methanol and milliQ water (25:75 v/v) before cooling and adjusting up to volume. The formoterol determination was performed using a validated analytical method.

2.2.2.3. Packaging and storage. Amounts of 24 ± 1 mg of each blend, corresponding to 12 µg of formoterol, were weighed manually in each type of capsule at the same time to be used for the same aerodynamic characterization test. This nominal dose and formoterol proportion in the powder correspond to the formoterol dosage and proportion encountered in inhaled medicines on the market (e.g. Foradil®, Formagal®) (Wauthoz et al., 2017). The filled capsules were conditioned in closed low density polyethylene containers for a minimum of three weeks at 20 °C and 50% RH in Pharma 600 chamber WeissTechnik® (Liederkerke, Belgium), which corresponds to the usual environmental conditions used by pharmaceutical companies for the production and storage of dry powders for inhalation.

2.2.2.4. Study protocol of aerosol characterisation. The aerosol performances of the blends filled into the different capsules were evaluated using the recommended airflow corresponding to a pressure drop of 4 kPa through the device for inhalation. This was adjusted to an airflow of 100 L/min for the low-resistance Axahaler DPI,

Table 2

Protocol study of aerosol performances describing the analysis done (uniformity of delivered dose, UDD; assessment of the fine particle dose, FPD; Aerosol particle size distribution, Aerosol PSD) for all the capsule type at the same time. UDD1-3 means that 3 delivered doses for each capsule filled with one blend were evaluated the same day for the 5 capsule type (HGC and QV for gelatine capsules, VC and QVI for cold-gelled HPMC capsules and VC+ for thermal-gelled HPMC capsules).

Day of the analysis/capsule type	T1	T2	T3	T4	T5	T6	T7	T8
HGC	UDD 1–3	FPD 1	UDD 4–5	FPD 2	UDD 6–7	FPD 3	UDD 8–10	Aerosol PSD 1–5
QG								
VC								
QVI								
VC+								

as recommended by the European Pharmacopeia (Pharmacopeia, 2012). The duration applied (i.e. 2.4 s) corresponded to 4 L of air passing through the device at this airflow. A mouthpiece adaptor for the Axahaler DPI was used, designed by Copley (Copley Scientific Limited, Nottingham, UK). The airflow was generated and controlled by a TPK2000 critical flow controller with two HCP5 high capacity pumps (Copley Scientific Limited, Nottingham, UK) to ensure a critical flow during analysis ($P3/P2 \leq 0.5$). A new DPI was used for each kind of capsule filled with each blend. The aerosol PSD, uniformity of delivered dose (UDD), and aerodynamic evaluation of fine particle dose (FPD, $< 5 \mu\text{m}$) using a next generation impactor (NGI) (Copley Scientific Limited, Nottingham, UK) were determined as reported in Table 2. The same determination was made on the same day for the five types of capsule at room temperature and relative humidity by the same technician. This methodology was done to evaluate strictly the influence of the capsule on the aerodynamic performance of the blend and eliminate other possible bias (e.g. environmental conditions, technician manipulation, repeated use of the DPI, etc.) (Mitchell, 2013).

2.2.2.4.1. Particle size distribution of the aerosol. The aerosol PSD was determined on five doses (Table 2) after aerosol generation in an inhalation cell coupled to a Spraytec® laser diffraction-based apparatus (Malvern Instruments, Worcestershire, UK), as described in Pilcer et al. (Pilcer et al., 2008). The aerosol PSD was characterized by its median diameter (Dv50), the diameter below which is the diameter of 90% of the particle volume (Dv90), and the mean mass diameter (D[4,3]).

2.2.2.4.2. UDD. The UDD of both dry powders packaged in the different kinds of capsule was determined using a dosage unit sampling apparatus (Copley Scientific Limited, Nottingham, UK) containing a 47 mm glass fibre filter (Pall Corporation®, USA) on 10 dosage units.

Each delivered dose (i.e. the formoterol dose emitted from the capsule and the device) was recovered from the dosage unit sampling apparatus with the previously described dilution phase in a 50-mL volumetric flask, sonicated for 20 min, and filtered on a 25 mm glass microfibre filter (GE Healthcare Life Science, UK). The formoterol determination was performed using a validated analytical method.

2.2.2.4.3. FPD and capsule retention. The aerodynamic behaviour of the dry powders packaged in the different capsules was determined using an NGI (Apparatus E, Eur. Ph. 8.0) (Copley Scientific Limited, Nottingham, UK) with a pre-separator and uncoated plates. Three independent tests were performed on the capsules. For each test, 10 prefilled and stored capsules, as mentioned in “Packaging and storage”, were used. The quantity of formoterol deposited at each level, i.e. capsules, device, induction port, pre-separator, stages 1–7, and micro-orifice collector (MOC), was recovered using the previously described dilution phase. The quantity of formoterol deposited was determined using a validated analytical method. The FPD corresponding to the dose of particles presenting an aerodynamic diameter below $5 \mu\text{m}$ was determined using the Copley inhaler testing data analysis software (Copley Scientific Limited, Nottingham, UK).

2.2.2.5. Analytical method. The determination of formoterol was performed using a validated method applied on a chromatographic system (HP 1200 series, Agilent Technologies, Brussels, Belgium)

equipped with a binary pump, an auto-sampler, and a diode array detector. The separations were performed on a LiChrospher 60 RP Select ($125 \times 4 \text{ mm}$, $5 \mu\text{m}$) column equipped with a pre-column (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile and phosphate buffer 0.01 M, pH 2.7 (25:75 v/v) at 1.0 mL/min. The quantification was performed at 245 nm. The standard curve was linear in the 25–1000 ng/mL range, with a limit of quantification (LOQ) of 25 ng/mL and limit of detection (LOD) of 7.5 ng/mL. The samples were recovered and diluted with the dilution phase consisting of methanol and milliQ water (25:75 v/v). The volume injected was 200 μL using a 400 μL extension loop (Agilent, Brussels, Belgium). The temperature was set at 30 °C and the analysis time was 6 min.

2.2.2.6. Data analysis. Statistical comparisons of two independent groups or more than three independent groups of data were made using a Student’s *t*-test or one-way analysis of variance (ANOVA), respectively. Before carrying out these tests, the homoscedasticity of variance was checked using Fisher’s exact test ($n = 2$) or Cochran’s C test ($n > 2$). When the multi-group test was significant, post-hoc tests (Newman-Keuls Multiple comparison) were used to avoid multiple comparison effects when comparing the group pairs of interest. When homoscedasticity of variances was not encountered, a non-parametric Kruskal Wallis test (KW) was performed with a Dunn’s multiple comparison test. In the results, NS, *, **, and *** correspond to $p > 0.05$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

3. Results

3.1. Aerodynamic performance of blends packaged in different capsule types

3.1.1. Binary and ternary blends

The formoterol content for each single dose of the binary and ternary blends was $11.96 \pm 0.14 \mu\text{g}$ (CV of 1.2%) and $11.59 \pm 0.33 \mu\text{g}$ (CV of 2.8%), respectively. In addition, none was outside the limits of 85% and 115% or the limits of 75% and 125% of the average content of the test for uniformity of content of single-dose preparations (Test B) in European Pharmacopeia 8.0 and as the CV% of the drug content was below 5%, the blend was considered homogeneous (Pilcer et al., 2012).

3.1.2. Aerosol PSD

No significant difference was observed between the Dv50, Dv90, and D[4,3] for the capsule type for both blends (NS, ANOVA) (Fig. S2 in supplementary data).

3.1.3. UDD

Fig. 1 reports the results of the delivered doses (i.e. the formoterol dose emitted from the capsule and the device) for each capsule type in the binary and the ternary blends.

For the binary blend, delivered doses from the different capsules were not significantly different except for the delivered dose from the QVI (*), which was significantly higher than the delivered doses from

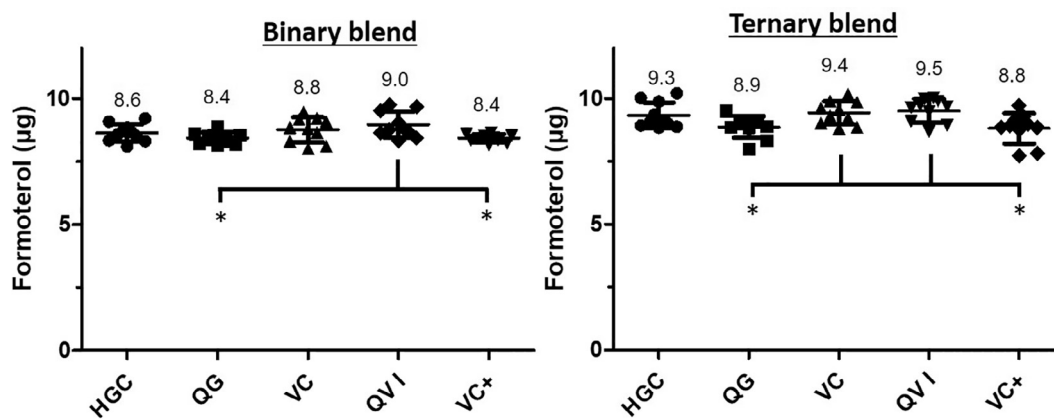


Fig. 1. UDD from the Axhaler DPI loaded with each capsule type (gelatine: HGC, QG; cold-gelled HPMC: VC, QVI; and thermal-gelled HPMC: VC+), prefilled with the binary mixture or the ternary mixture. Each dot represents a single data point, with the bar for the mean ± SD (n = 10). The mean value is indicated above the dots and * corresponds to p < 0.05.

the VC+ and QG (ANOVA). For the ternary blend, the trend was similar but with the fact that the delivered doses from the VC (*) was also significantly higher than the delivered doses from the VC+ and the QG (ANOVA).

3.1.4. FPD and capsule retention

Figs. 2 and 3 report the results for FPD (i.e. the formoterol dose showing an aerodynamic diameter < 5 µm and able theoretically to deposit into the lungs) and capsule retention (i.e. the formoterol dose recovered from the capsule after the dose delivery and expressed as a percentage related to the nominal dose) for each capsule type in the binary and the ternary blend, respectively.

For the binary mixture, the FPD from the HGC and VC+ and the FPD from the VC and QVI were non-significantly different (NS, ANOVA). The FPD from the QG (***), HGC (**), and VC+ (**) were significantly lower than the FPD from the QVI and VC (ANOVA). The FPD from the QG (*) was significantly lower than from the HGC and VC+ (ANOVA). For the ternary mixture, the trends were similar but the FPD values were much more closer to those obtained for the binary mixture with a lower significant difference between the FPD values from the QG (**) and the FPD from the QVI and VC (ANOVA). Moreover, there was no significant difference between the FPD from the HGC, VC+, VC, and QVI (NS, ANOVA).

For the binary mixture, differences in the formoterol retention from the different capsules were not significant between the VC and QVI, between the QG and HGC, and between the VC+ and gelatine capsules (HGC and QG) (NS, ANOVA). The formoterol retention was

significantly higher for the HGC (**), QG (* or **) and VC+ (**) in comparison with the VC and QVI (ANOVA).

For the ternary mixture, formoterol retention from the different capsules showed the similar trends but with much more differences between the obtained results in comparison with those from the binary blend.

However, the differences were non-significant except for the HGC (*), which was significantly higher than formoterol retention from the QVI (KW). In fact, the HGC showed a high variability in capsule retention, which led to a variance in heterogeneity. This variance led to the use of a lower-discriminating statistical test (i.e. the KW non-parametric test).

3.1.5. In vitro deposition in the NGI

The in vitro deposition profiles in the NGI for all capsules for the binary mixture and ternary mixture are expressed as the percentage of the nominal dose and are reported in Fig. 4.

In comparison with HPMC capsules (i.e. the VC, QVI and VC+), gelatine capsules (i.e. the HGC and QG) showed a high increase in deposition in the pre-separator for both blends and a slightly higher deposition in the induction port for the binary blend. This increased deposition led to a lower deposition for gelatine capsules in stages S3 to the MOC for the binary mixture and in the stage S5 to the MOC for the ternary blend. Once we compared cold-gelled HPMC capsules (i.e. VC, QVI) with thermal-gelled HPMC capsules (i.e. VC+), the cold-gelled HPMC capsules showed a slight decrease in formoterol deposition in the pre-separator for both dry powders, and a higher increase in deposition

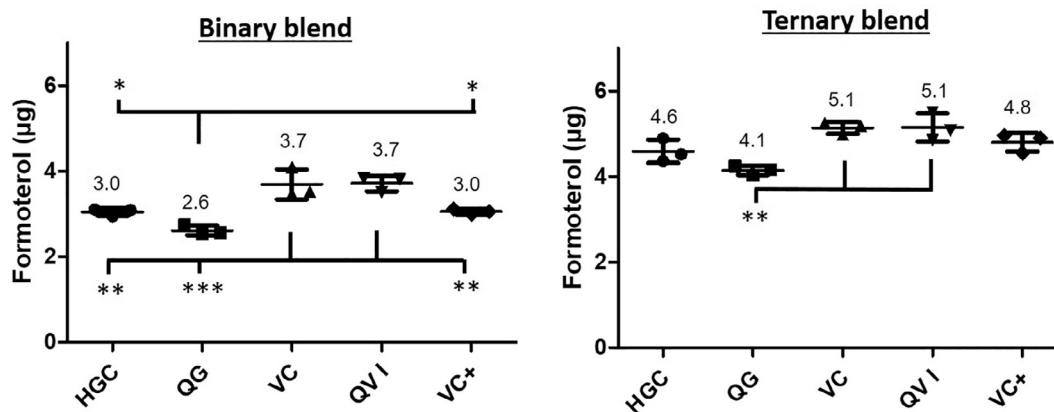


Fig. 2. FPD from the Axhaler DPI loaded successively with 10 capsules of each type (gelatine: HGC, QG; cold-gelled HPMC: VC, QVI; and thermal-gelled HPMC: VC+), prefilled with the binary mixture or the ternary mixture. Each dot represents a single data point, with the bar for the mean ± SD (n = 3). The mean value is indicated above the dots. *, **, and *** correspond to p < 0.05, p < 0.01, and p < 0.001, respectively.

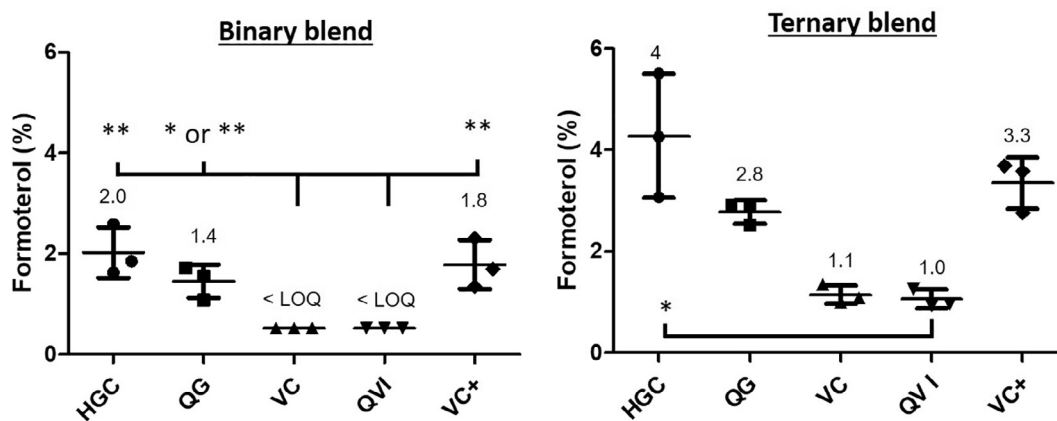


Fig. 3. Formoterol capsule retention from the Axahaler DPI loaded successively with 10 capsules of each type (gelatine: HGC, QG; cold-gelled HPMC: VC, QVI; and thermal-gelled HPMC: VC+), prefilled with the binary mixture or the ternary mixture. Each dot represents a single data point, with the bar for the mean \pm SD (n = 3). The mean value is indicated above the dots. A mean value below the limit of quantification is indicated as < LOQ and corresponded to LOQ/2 (i.e. 0.52%). * and ** correspond to $p < 0.05$ and $p < 0.01$, respectively.

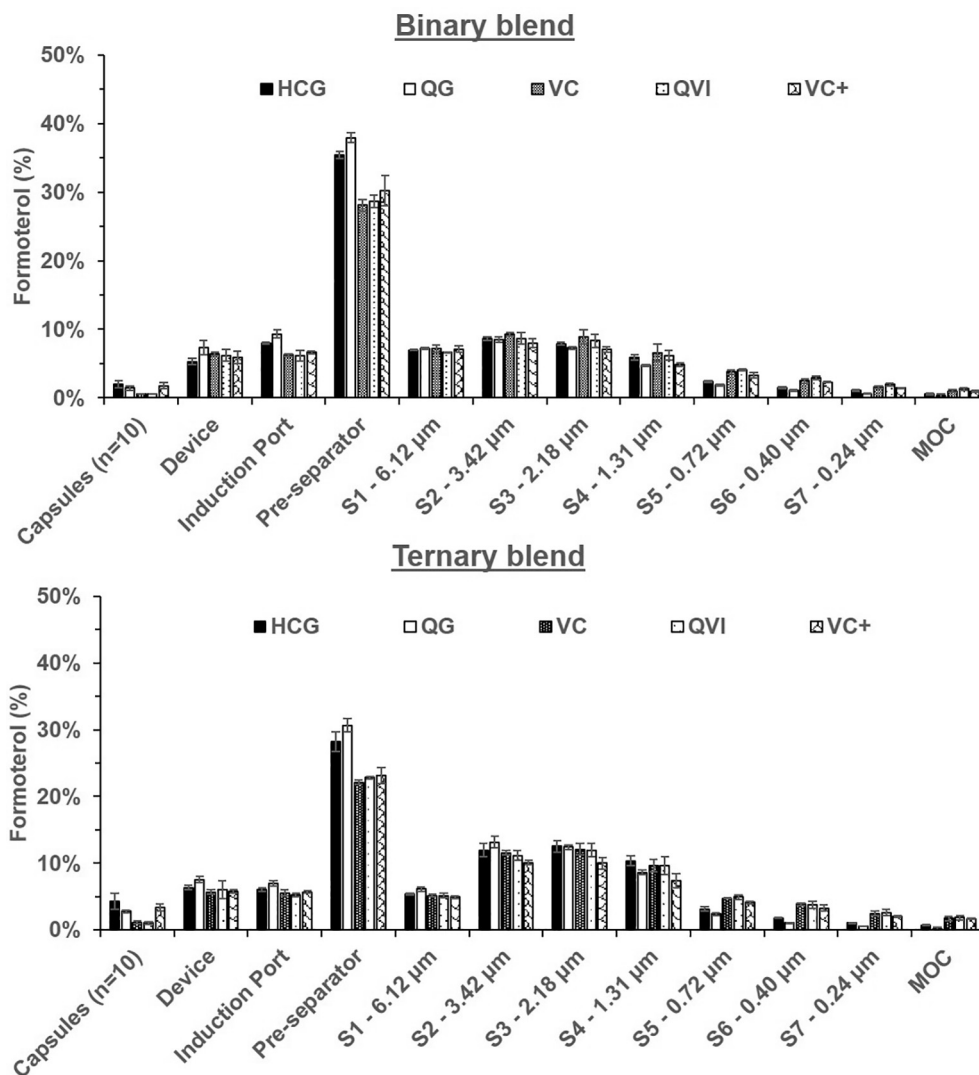


Fig. 4. *In vitro* deposition in the NGI from the Axahaler DPI loaded successively with 10 capsules of each type (gelatine: HGC, QG; cold-gelled HPMC: VC, QVI; and thermal-gelled HPMC: VC+), prefilled with the binary mixture or the ternary mixture. The results are represented as a percentage related to the nominal dose and expressed as a mean \pm SD (n = 3).

Table 3

Water content of each capsule type determined by TGA (weight loss between 27 °C and 180 °C), and characterization of the surface roughness and of craters observed on the inner surface of each capsule type by confocal microscopy.

Capsule	HGC	QG	VC	QVI	VC+
Water content (%)	13.2 ± 0.1	13.0 ± 0.1	4.10 ± 0.08	4.4 ± 0.10	4.24 ± 0.04
Mean ± SD					
Surface roughness (Sa/μm)	0.05 ± 0.06	0.07 ± 0.10	0.06 ± 0.08	0.06 ± 0.09	0.06 ± 0.09
Mean ± SD					
Total volume craters (V/μm ²)	0.009	0.021	0.024	0.025	0.023
Mean ± SD					
Average diameter of crater	N.D.	1.09 ± 1.01	0.76 ± 0.39	1.09 ± 1.04	1.51 ± 1.50
Mean ± SD					
Average depth of crater (h/μm)	N.D.	0.17 ± 0.12	0.28 ± 0.29	0.24 ± 0.15	0.21 ± 0.16
Mean ± SD					

N.D. not determined.

in stages S2 to the MOC.

3.2. Empty capsule characterization

The water content determined by TGA and the characterization of the inner surface of each capsule determined by confocal microscopy are reported in Table 3. SEM pictures of the different hole types observed and of the outer and inner surface of each capsule type are

reported in Fig. 5.

The water content in the capsules was in the lower part of their normal specification. Gelatine capsules contain between 13.0 and 16.0% water and HPMC capsules between 4.5 and 6.5% for inhalation grade. However, the water content of the gelatine capsules was much higher than the water content of the HPMC capsules, with no difference between cold-gelled or thermal-gelled HPMC capsules. Moreover, the TGA profiles were quite different for gelatine capsules and HPMC

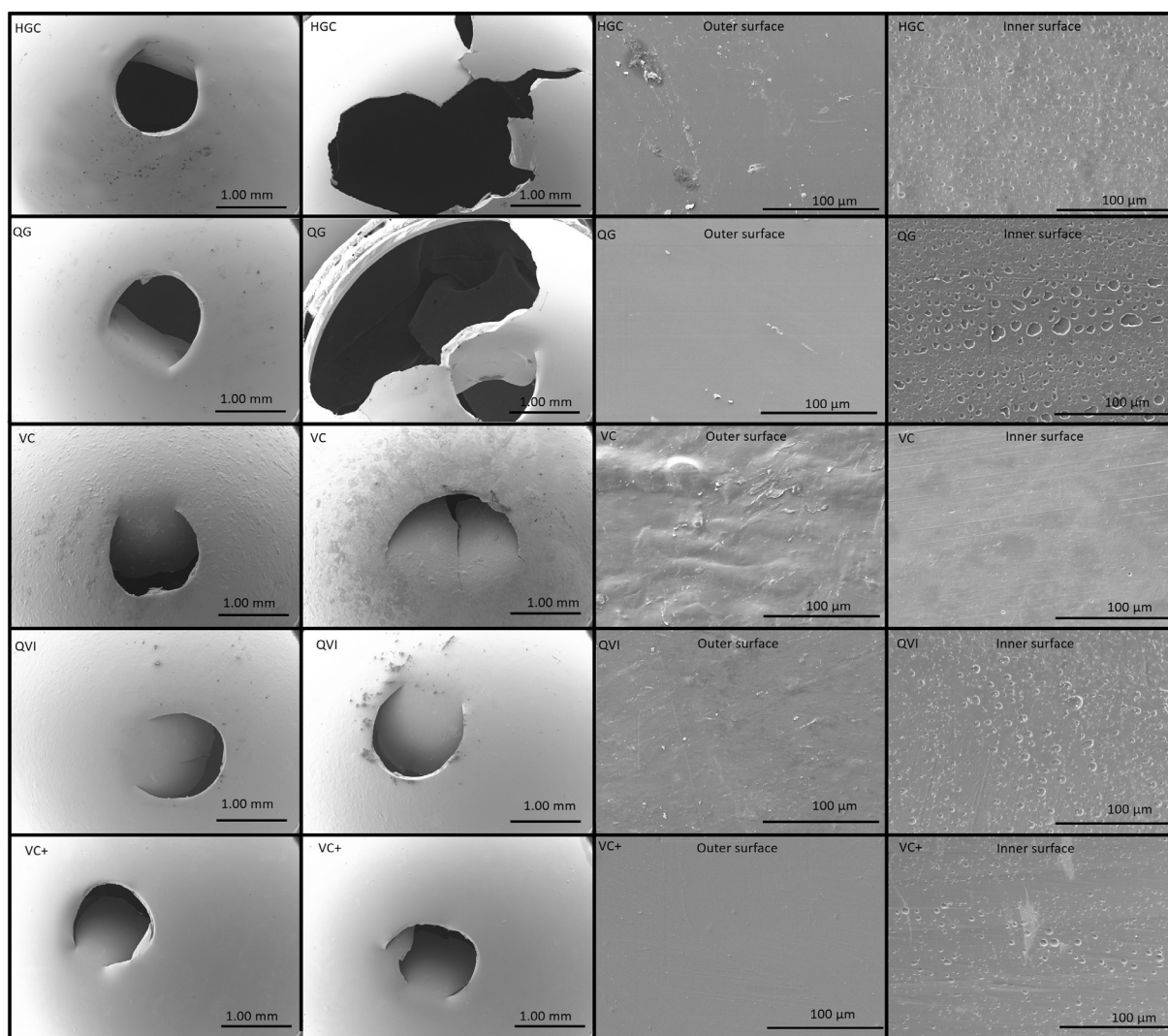


Fig. 5. SEM pictures of hole type after piercing and inhaling at 100 L/min through the Axahaler DPI (magnification of 30 ×) and of the outer and inner surface of the capsule body (magnification of 500 ×).

capsules as water is strongly bound in gelatine capsules (Fig. S3 in the supplementary data). The inner surface of the capsules was quite different to the outer surface, as observed in Fig. 5. Some craters are observed in the inner surface. The diameter of these craters corresponds to the diameter of grease droplets recovered on the dipping pin surface (i.e. a lubricant print). The surface properties are directly linked to the depth, diameter, and total volume of craters (Table 3). Moreover in Fig. 5, gelatin (i.e. QG and HGC) and thermal-gelled HPMC (VC+) capsules showed smoother surface than cold-gelled HPMC capsules. In terms of hole aspect after piercing and inhaling procedure, no HPMC capsules showed cracked or enlarged holes in comparison to gelatin capsules (Fig. 5). For gelatin capsules, about 30% showed larger and irregular holes (3–4 times the regular hole size presenting a 1.0 mm diameter) (Fig. 5).

4. Discussion

Aerodynamic performances of a dry powder result from many interconnected factors including the patient/technician, the device (e.g. resistance, design), the formulation (i.e. micronized drug, lactose carriers), the capsule in the case of capsule-based DPIs, the airflow, the humidity, etc (Mitchell, 2013; Schoubben et al., 2015; Martinelli et al., 2015). However, there are few studies evaluating the impact of the capsule type on aerodynamic performance. In one of them, it was shown by a statistical analysis that the capsule brand (two types of HPMC capsule) and the formulation (two types of formulation) have a significant impact on the fine particle fraction related to the emitted dose or nominal dose with the highest impact for the formulation ($p \leq 0.001$) followed by the capsule brand ($p \leq 0.01$ or $p \leq 0.05$ for the fine particle fraction related to the emitted dose or to the nominal dose, respectively), and low or no impact of the capsule-based device (three types of capsule-based DPI, $p \leq 0.05$ or $p > 0.05$ for the fine particle fraction related to the emitted dose or nominal dose, respectively) (Schoubben et al., 2015). Therefore, in this study, the aim was to investigate more the capsule type and brand by evaluating five capsules using two different formulations and only one device and that at its recommended airflow. Each aerodynamic characteristic was evaluated with all the different capsule type by the same technician and at the same time in the aim to evaluate rigorously and deeply only the impact of the capsule type. The aim was to determine if there are some statistical differences only attributed to the capsule type and if these trends are confirmed in two different dry powder formulations (i.e. a binary or a ternary blend elaborated using the same micronized drug batch).

Aerodynamic performance was evaluated for a binary mixture and a ternary mixture packaged in different kinds of capsules (QG and HGC for gelatine capsules, QVI and VC for cold-gelled HPMC capsules, and VC+ for thermal-gelled HPMC capsules), and that at the recommended airflow by the European Pharmacopeia. The aerodynamic performance was evaluated in terms of delivered dose FPD, and formoterol capsule retention, as shown in Figs. 1–3, respectively. Moreover, the *in vitro* deposition in the capsules, device, and all parts of the NGI is shown in Fig. 4 for both blends to better understand the observed differences.

Similar trends were observed for the capsule retention, delivered doses, and FPD extrapolated from the *in vitro* deposition in the NGI for each capsule type (i.e. gelatine, cold-gelled HPMC, and thermal-gelled HPMC capsules) for both blends. For both blends, the highest delivered dose and FPD and the lowest formoterol capsule retention were observed with cold-gelled HPMC capsules such as the QVI and VC, with no significant differences between them. Similar emitted fraction and fine particle fraction related to the nominal dose results were observed using cold-gelled HPMC capsules (Quali-V and Vcaps) with an antibiotic blended with lactose carrier using the same RS01 model (Schoubben et al., 2015). However, it is often dependent on the kind of formulation as a spray-dried formulation using these two capsules have shown drastic differences with better performance (i.e. fine particle fraction related to the nominal dose) for Quali-V[®]-I due to much higher emitted

fraction.

There was a similar deposition in the device. Therefore, the higher delivered dose in cold-gelled HPMC capsules is explained by the lower formoterol retention in these capsules in comparison with gelatine or thermal-gelled HPMC capsules.

The higher FPD in cold-gelled HPMC capsules is explained by a much lower deposition in the pre-separator in comparison with gelatine or thermal-gelled HPMC capsules, leading to higher depositions in the stages presenting a cut-off below 5 μm (i.e. mainly in the stages S2 to the MOC). In both blends, the thermal-gelled HPMC capsules presented very close aerodynamic performance (delivered dose, FPD, and capsule retention) as HGC capsules.

To understand the reasons for these differences, the water content, morphology of the hole and the outer and inner capsule surface, and the characterization of inner surface were assessed for each capsule type (Table 3 and Fig. 5).

The difference in aerodynamic performance (capsule retention, delivered dose, and FPD) observed between gelatine capsules (HGC, QG) and cold-gelled HPMC capsules (QVI, VC) can be explained by the higher water content in the capsule shell (13% in comparison to 4%, Table 3). In this case, water from the gelatine shell can increase adhesion forces by capillary forces between the micronized drug and the inner surface of the capsule, leading to higher formoterol capsule retention. Moreover, water from the gelatine shell can also be transferred from the capsule shell to the powder (Barham et al., 2015). Water transfer can increase adhesion forces between the micronized drug and the lactose carrier or fine lactose particles by capillary forces, leading to less detachment of the micronized drug from the coarse carrier or less drug-drug and/or drug-fine lactose de-agglomeration. Reduced detachment and increased attachment to drug and/or to fine lactose particles increases drug deposition in the pre-separator and decreases deposition in stages presenting a cut-off $< 5 \mu\text{m}$ (Pilcer et al., 2012). This issue is reduced in the ternary blend as micronized lactose particles form agglomerates with the micronized drugs during the blending and/or act in competition with the micronized drugs for the active sites of the coarse carrier (Pilcer et al., 2012). This led to a lower increase of particle deposition in the pre-separator and a decrease in the deposition more deeply in the NGI (from particles presenting a size $< 1.31 \mu\text{m}$) in comparison with the binary blend (Fig. 4). Moreover, this hypothesis is supported by the similar aerosol PSD observed by a Spraytec[®] laser diffraction-based apparatus for all capsule types for each blend (Fig. S2 in supplementary data). As the aerosol PSD results show, the PSD of aerosolized particles mainly composed of coarse lactose, similarities reveal that the agglomeration is not between coarse lactose particles but between coarse lactose and micronized particles (drug or fine lactose).

Moreover, during the study, about 30% of gelatine capsules (HGC, QG) were cracked or fractured after the piercing and inhalation procedure. This damage led to much larger and more irregular holes (3–4 times the regular hole size presenting a 1.0 mm diameter) than with the HPMC capsules, where 0% cracking and fracturing was observed (Fig. 5). When the capsule shell is punctured in the DPI, the holes produced need to be regular in shape and size. The material cut from the wall should remain attached as a flap and remain in an open position when the needles are removed (Birchall et al., 2008). The irregular holes for the gelatine capsules seem not to be related to the brittleness of gelatine capsules at low humidity levels, as discussed before. Due to the fact packaged capsules were maintained at 20 °C and 50% RH for 3 weeks before the analysis. Furthermore, the aerodynamic analyses were performed at room temperature and humidity (i.e. $21 \pm 1 \text{ }^\circ\text{C}$ and $48 \pm 9\% \text{ RH}$ for the binary blend and $21 \pm 1 \text{ }^\circ\text{C}$ and $38 \pm 6\% \text{ RH}$ ($n = 225$ and 200) for the ternary blend). The incidence of cracked or fractured capsules does not seem to correlate to the relative humidity recorded during the analysis or previous handling but seems to be explained by the capsule material (i.e. gelatine vs HPMC). Despite a lower resistance to deformation, HPMC capsules maintain

their puncturing properties or resistance to breakage over a wider range of humidity than gelatine capsules, which could explain these differences (Birchall et al., 2008; Chong et al., 2016).

An increased hole size can decrease the capsule's de-agglomeration ability, as demonstrated by Coates et al. for the Aerolizer[®] DPI or modified Aerolizer DPIs with different number of pins and/or with different pin diameters. The size of the capsule hole affects powder dispersion. A small capsule hole size increases the break-up mechanism by forcing powder agglomerates through the capsule hole (Coates et al., 2005). In Coates's study, a single 1.0 mm hole size gave a better FPD related to the nominal dose than a single 1.5 mm hole size (43.7% vs 40.0%, respectively) (Coates et al., 2005). However, this improvement cannot be related directly to a smaller total hole(s) area as four 0.6 mm holes give a better FPD than a single 1.0 mm hole (54.9% vs 43.7%, respectively) (Coates et al., 2005).

The lower aerodynamic performances (higher capsule retention, lower delivered doses, and lower FPD) observed with thermal-gelled HPMC capsules (i.e. VC+) in comparison with the cold-gelled HPMC capsules (i.e. VC and QVI) in the binary blend can be explained neither by the water content of the capsules (about 4% for all of them, Table 3) nor by the hole diameter (Fig. 5). The last important factors that may have an influence are the properties of the inner surface of the capsules. The inner surface comprises the lubricant content and the capsule shell. This combination presents a specific physical and chemical composition, leading to different surface properties in terms of morphology, static electricity, and surface energy. The lubricant content has been shown to influence aerodynamic performance in terms of capsule retention, delivered dose, and FPD (Ayala et al., 2016; Saleem et al., 2015). It was shown that a minimal lubricant content (> 60 ppm) was necessary to decrease the surface roughness of the inner surface and therefore decrease the capsule retention and increase the delivered doses and FPD. However, in this case, the lubricant content of the VC+ is quite similar to the VC (213 ppm vs 227 ppm, Table 1) and there are no drastic differences in the surface roughness or crater evaluations (Table 3). Confocal microscopy analysis revealed similar surface roughness and total volume of craters per surface, and only slightly larger and lower deep craters for the VC+ in relation to the VC and QVI, Table 3.

One important factor that could explain the difference between thermal-gelled HPMC capsules and cold-gelled HPMC capsules is the behaviour of a different chemical composition as the thermal-gelled HPMC capsules do not contain a gelling agent or a gelling promoter. Moreover, the process for producing thermal-gelled HPMC capsules is drastically different than the process for producing cold-gelled HPMC capsules. The process involves heating the dipping pins for thermal-gelled HPMC capsules (Jones et al., 2004). Heating the dipping pins can influence the physicochemical properties of the lubricant and/or of the capsule shell, leading to changes in surface properties (e.g. static electricity, surface energy, morphology). Some changes are visible on the capsule shell as the outer surface was shown to be less uniform for cold-gelled HPMC capsules (i.e. VC and QVI) than for thermal-gelled capsules (i.e. VC+) (Fig. 5). The outer surface of thermal-gelled HPMC capsules was closer to the smooth morphology of gelatine capsules (i.e. HGC and QG) (Fig. 5). This could partly explain the greater similarity between the aerodynamic performance of thermal-gelled capsules and gelatine capsules, as observed between the VC+ and the HGC (Figs. 1–3).

The aim of this study was to evaluate strictly the influence of the capsule type on the aerodynamic performance of dry powders using statistical analysis and to avoid possible bias. To achieve this, all capsule types (provided from the same batch) were evaluated in the same day for the same analysis with the same technician. Therefore, the evaluation of the two blends was not done on the same day but in two successive phases, respecting the same methodology. However, some observations can be made. Independently of capsule type, the ternary blend showed a significantly higher delivered dose and FPD despite a

significantly higher capsule retention in comparison to the binary blend (***) for the delivered dose and FPD and ** for capsule retention, Student's *t* test). This is due to a lower deposition in the device for the delivered dose and in the pre-separator for the FPD (Fig. 4). This can be explained by the method and the carriers used to make the blends. The ternary blend was made using coarse lactose with a narrow PSD, and micronized lactose was added at the same time as the micronized drug for blending. In comparison, for the binary blend, coarse lactose with a broad PSD that included fine lactose particles was used, before blending it with the micronized drug. As micronized lactose and drug have a relatively comparable size, they can form agglomerates that are able to adhere onto active sites of coarse lactose. Moreover, the micronized lactose can compete with the micronized drug to adhere onto certain active sites that present higher adhesion than others. In the binary blend, the fine lactose already existed in the coarse lactose powder (Fig. S1 in the supplementary data). This fine lactose, already fixed onto the active sites of the coarse lactose, either could not form agglomerates with the micronized drug or did so to a much lower extent than in the ternary blend. Therefore, during inhalation, agglomerates detached more easily from the coarse carrier in the ternary blend than the micronized drug from the carrier in the binary blend. This easier detachment led to a much lower impaction of micronized drug in the pre-separator for the ternary blend. The higher mass of agglomerates presented a higher inertia, which led to a better deagglomeration/dispersion by the forces derived from the inspiratory airflow than the micronized drug alone (Pilcer et al., 2012). This implies that the micronized drug can reach the later stages of the impactor more deeply. However, agglomerates of fine lactose and micronized drug can retain a higher content of the drug particles in the capsules, as observed in Fig. 3.

5. Conclusion

The capsule type showed a significant impact on the aerodynamic performance of two kinds of dry powder (binary or ternary mixtures). The lowest formoterol capsule retention and the highest delivered dose and FPD were observed for the cold-gelled HPMC capsules (i.e. VC and QVI) in comparison to gelatine (i.e. HGC, QG) or thermal-gelled HPMC (i.e. VC+) capsules. The differences in the aerodynamic performances between gelatine and cold-gelled HPMC capsules seemed to be attributed to the difference in water content and the proportion of the capsule that is cracked or fractured during the puncturing and inhalation step, which increases drastically the hole diameter. For the difference between cold-gelled and thermal-gelled HPMC capsules in aerodynamic performance, neither the water content, the hole diameter, the lubricant content, nor the inner surface morphology can explain these differences. However, the chemical composition and the manufacturing processes are drastically different for thermal-gelled HPMC capsules and cold-gelled HPMC capsules. For example, heating the dipping pins can influence the physicochemical properties of the lubricant and/or of the capsule shell, leading to changes in surfaces properties (e.g. static electricity, surface energy, surface morphology). These differences in surface properties could explain the differences observed in aerodynamic performance. Now, the aim is to expand this study by evaluating these capsule types at different airflows or at different environmental conditions to determine if a capsule type is more robust than others to factors linked to patients and, finally we will study these capsules using another drug presenting a higher drug dosage.

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This work was financially supported by Qualicaps Europe. The sponsor of this study approved the study design and was involved in the generation of confocal microscopy data. It was not involved in the interpretation of the data or in the writing of the article. The sponsor has read and approved the content of this article before its submission.

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Appendix A. Supplementary data

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