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In vitro evaluation of powders for inhalation: The effect of drug concentration on particle detachment

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ABSTRACT

Limited information on the effect of the drug concentration on the performance of powders for inhalation is currently published. The aim of this work was to study the influence of drug concentration on the adhesion between drug and carrier and on the drug detachment from the carrier. The study was done with formoterol fumarate and fluticasone propionate blended with lactose Lactohale 200. To assess the adhesion of respirable-sized drug to carrier particles, a simple method was developed based on aspiration and considering the whole blend as it is used in dry powder inhalers. Adhesion characteristics were evaluated by submitting the mixtures to a sieving action by air depression with an Alpine air-jet sieve. Aerodynamic evaluation of fine particle dose and emitted dose was obtained using a Twin Stage Impinger (TSI). Drug concentration of powder blends used in dry powder inhalers influenced adhesion, content uniformity and in vitro deposition of the drug. For the higher concentration of formoterol, it seemed that a lower quantity of drug adhered to the lactose. This was confirmed by the aerosolization assays done in the TSI. The fine particle fraction increased linearly with the formoterol concentration. A correlation was observed between adhesion characteristics and inertial impaction. In the case of fluticasone, the influence of the concentration was different. First, the fine particle fraction increased with the concentration and then decreased with a further increase of the fluticasone concentration. This could be explained by the lack of homogeneity when the fluticasone concentration was high because of agglomerates of pure drug which can not be redispersed, or by the physico-chemical characteristics of this drug.

1. Introduction

Dry powder formulations for inhalation are often composed of fine drug particles and inert coarse carrier particles, typically alpha monohydrate lactose. The fine drug particles that exhibit greater cohesiveness and adhesiveness are expected to adhere to the carrier surface to form adhesive mixtures. Interactions between particles are mainly dependent on the physicochemical characteristics of the interacting particles, that is to say: particle size, shape, surface morphology, contact area, hygroscopicity (Bell, 1994; Gupta and Hickey, 1991; Prime et al., 1997). During the mixing process, the adherence of drug particles to the more adhesive areas of the carrier surface is likely to occur. When the number of fine particles in the blend is below the saturation limit of the large particle adhesive potential, the fine particles will preferentially bind to these active sites. When these active sites have been completely occupied with fine particles, a binary carrier system would then exist, i.e., carrier with strongly bound fine particles, and free or weakly bound fine particles (Young et al., 2005). The retention of drug particles on these relatively high-energy sites during aerosolization would decrease the fine particle fraction (Staniforth, 1996). Particle interactions are of great importance in dry powder inhaler formulation where the redispersion of drug particles from carrier particles is critical for lung deposition. In such preparations, the inspiratory force of the patient must overcome the adhesion forces between drug and carrier particles to aerosolize particles.

The interactions may also be influenced by the drug load (De Boer et al., 2005; Steckel and Müller, 1997). Limited information on the effect of the drug concentration is currently published. Most studies on adhesive mixtures for inhalation focus only on a single drug concentration, for example 1.46% (w/w) for salbutamol or terbutaline sulphate with an administered dose of drug around 500 μg (Timsina et al., 1994; Zeng et al., 2000). More recently, the development of dry powder inhalers which can deliver lower doses of drugs has received attention. In this case, the drug has a higher potency and consequently requires a lower dose (e.g. formoterol fumarate 6–12 μg). This may lead to variations in drug delivery as a consequence of drug retention in high-energy sites resulting in

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severe deviations from target doses and label claims (Young et al., 2005). So it is of interest to consider adhesive mixtures with different drug concentrations and in particular at much lower ranges.

The aim of this work was to study the influence of the drug concentration on the adhesion between drug and carrier and on the drug detachment from the carrier. Our study was carried out with two different model drugs: formoterol fumarate and fluticasone propionate blended with Lactohale 200 used as the carrier. These two drugs are generally used in different dose ranges, so they will be tested in different concentration ranges. The formoterol fumarate which is administered at a low dose, that is to say 6 μg or 12 μg, will be tested at concentrations in the range of 0.09–0.17% (w/w). The fluticasone propionate requires higher dose, 100–500 μg, and will be tested at concentrations between 1.5% and 3.5% (w/w). For each drug, the influence of concentration on the drug/carrier interactions and on the aerodynamic behaviour was determined.

2. Materials and methods

2.1. Materials

Lactohale 200 (Friesland Foods Domo, Zwolle, The Netherlands) was used as carrier. The two drugs tested were micronised formoterol fumarate and micronised fluticasone propionate supplied by APTAR PHARMA (Le Vaudreuil, France).

The drug/carrier blends were aerosolized with the Inhalator Ingelheim (Boehringer Ingelheim, Ingelheim am Rhein, Germany) after filling in hard gelatine capsules (size 2 and 3 respectively for formoterol fumarate and fluticasone propionate) (Capsugel, Colmar, France).

2.2. Blending lactose with drugs

Lactohale 200 was mixed with 0.09%, 0.13% and 0.17% (w/w) of formoterol fumarate using a Turbula tumbling mixer (Bachofen Maschinenfabrik, Basel, Switzerland) at 74 rpm during 60 min. Lactohale 200 was also mixed with 1.5%, 2.5% and 3.5% (w/w) of fluticasone propionate in a Turbula tumbling mixer at 90 rpm for 180 min.

The blending conditions were the optimal conditions for each drug. Indeed, a previous work studied the influence of the operating conditions, in particular speed and mixing time, on homogeneity, adhesion and dispersion of the drug. We observed that for good dispersion and therefore good homogeneity of the drug in the carrier particles, speed and powder blending time have to be sufficient, but not too high for the speed to prevent the apparition of static electricity, which is not favourable to homogeneity and stability. A mixing time as long as 180 min for fluticasone propionate was necessary because of the cohesive nature of this drug and of the type of blender (Turbula) which is not high shear mixer.

Each blend was prepared in 100 g quantities.

2.3. Measurement of average content

The quality of the blends was examined by analysing the quantity of drug in aliquots of powder equivalent to the amount of powder in each capsule: 50 mg for formoterol blends, 20 mg for fluticasone blends. Fifteen aliquots were taken randomly from each blend and assayed using an UV spectrophotometer (UV-1650PC, Shimadzu, Kyoto, Japan) with a wavelength of 206 nm for the formoterol fumarate. A calibration curve was established from the concentration 0.1 μg/ml to 10 μg/ml ($A = 0.0866$, $R^2 = 0.9997$). For the fluticasone propionate, drug content was determined using HPLC (ProStar 230, Varian, Paris, France) with a Pursuit C18 column (150 mm × 4.6 mm, 5 μm) according to an internal method of APTAR PHARMA. The drug was detected by UV spectrophotometry at a wavelength of 236 nm.

From the 15 results of drug content in the samples, the average content of drug and the mean recovery related to the nominal dose were calculated. The relative standard deviation percentage (%RSD) was calculated and used to assess the homogeneity of the drug.

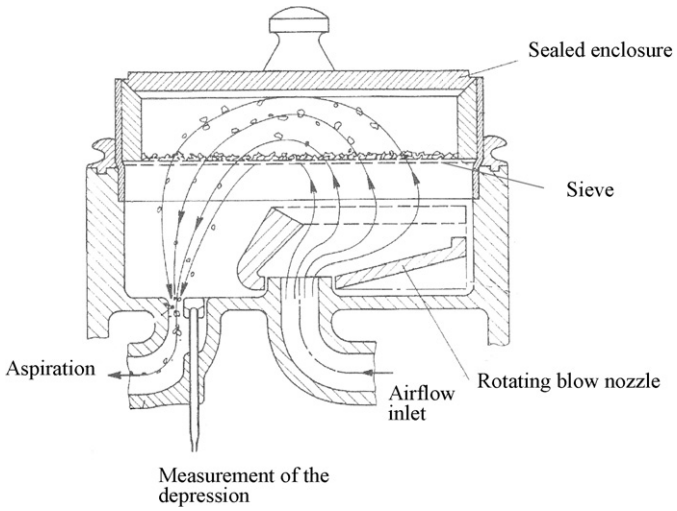

Fig. 1. Experimental setup for the air-jet sieving.

2.4. Evaluation of adhesion characteristics

To assess the adhesion of respirable-sized drug to carrier particles, a simple method was developed based on aspiration and considering the whole blend as it is used in dry powder inhalers. Adhesion characteristics were evaluated by submitting the mixtures to a sieving action by air depression with an Alpine air-jet sieve (Alpine, Haan, Germany) used with an airflow that produced a pressure drop of 4 kPa (Fig. 1). Blends were put on the 32 μm sieve section of the Alpine air-jet apparatus, in a sealed enclosure. Particles were submitted on the one hand to an airflow released by a blow nozzle rotating under the sieve and, on the other hand, to aspiration through the sieve. The drug particles that were detached from the carrier and suspended in air were carried through the sieve thanks to aspiration. The objective of this test was to assess the ease with which the drug can be separated from the carrier when the blend is carried by an airflow.

30 g of blend was placed on the 32 μm sieve section of the Alpine air-jet apparatus. Three samples of 50 mg (for formoterol) or 20 mg (for fluticasone) were removed from the powder bed after sieving for different lengths of time: 5, 30, 60 and 150 s. For each sample the percentage of remaining drug was compared to the initial dose, which is an indicator of the quantity of drug that adheres to the carrier. Indeed if the drug particles were separated from the carrier, they would be carried away through the sieve by aspiration.

The drug content in the samples was determined by UV spectrophotometry for the formoterol fumarate and by HPLC for the fluticasone propionate.

For all experiments conducted, temperature and relative humidity were controlled at 20 °C and 40–45%.

2.5. Preparation of the capsules

The Lactohale 200/drug blends were filled into hard gelatine capsules manually so that each capsule contained 50 mg of blend (49.79–50.21 mg, SD = 0.170) in the case of formoterol fumarate and

20 mg of blend (19.85–20.12 mg, SD = 0.125) in the case of fluticasone propionate.

2.6. Aerodynamic evaluation of fine particle dose and emitted dose

In vitro deposition of drug from dry powder formulations was determined using a twin stage impinger (TSI, Apparatus A, European Pharmacopoeia, 2008). The TSI was assembled and loaded with 7 ml and 30 ml of solvent (water/methanol 30/70% (v/v) in the case of fluticasone propionate and water in the case of formoterol fumarate) in the upper and lower stages respectively. Each deposition experiment involved the aerosolization at 60 l/min during 5 s via an Inhalator Ingelheim of 5 capsules containing 20 mg of blend in the case of fluticasone propionate, and 10 capsules containing 50 mg of blend in the case of formoterol fumarate. The different parts of the TSI were rinsed and the amount of drug deposited in the upper and lower stages was determined using spectrophotometry for the formoterol fumarate and using HPLC for the fluticasone propionate.

For each blend, the assays were performed in triplicate and the following parameters were used to characterize the deposition profiles of the drug:

- the emitted dose (ED), which is the sum of drug collected at upper and lower stages, divided by the number of capsules tested
- the fine particle dose (FPD) defined as the amount of drug deposited in the lower stages of the TSI, because their aerodynamic diameter was less than the cut-off diameter of the TSI (6.4 μm at an air-flow rate of 60 l/min), divided by the number of capsules tested
- the percentage emitted calculated as the ratio of ED to the average content
- the fine particle fraction calculated as the ratio of FPD to the emitted dose.

A parametric test of analysis of variance (one factor ANOVA) was applied to the results.

2.7. Industrial assays with the Prohaler®

Lactohale 200/fluticasone blends at concentrations of 2.5%, 3.5% and 5% were also filled into the Prohaler®, the new DPI device of APTAR PHARMA. The blister strips were filled so that there was 10 mg of blend in each blister. Dose Content Uniformity (DCU) was measured on 30 doses of the device. The fine particle fraction was determined with the NGI impactor. The emitted fraction was the mean of the emission of 15 doses.

3. Results and discussion

3.1. Influence of drug concentration on the average content and variability

Table 1 presents the percentage of recovery of drug in the different samples compared to the nominal dose for the different blends. For formoterol fumarate, when the percentage increased, the drug recovery increased. In fact, a quantity of drug was able to adhere to the container. Above a certain concentration, this quantity is probably the same whatever the formoterol concentration. So, if this quantity is related to the formoterol concentration, the loss will be greater in percentage when the drug concentration is lower. This is illustrated in Fig. 2 where a linear relationship between the mean content and the formoterol concentration has been observed with a correlation coefficient R^2 of 0.97 which indicated a good correlation between these two parameters.

Table 1
Average content in drug (% w/w) for the different blends.

Drug in the blend (% w/w)	Average recovery content (%)
Formoterol fumarate	
0.09	87.57 (RSD: 2.92)
0.13	94.10 (RSD: 5.9)
0.17	97.58 (RSD: 2.81)
Fluticasone propionate	
1.5	94.14 (RSD: 7.33)
2.5	96.97 (RSD: 5.62)
3.5	103.35 (RSD: 11.34)

The same tendency was observed with the fluticasone propionate which was used at higher concentrations (Table 1).

Table 1 shows drug homogeinity results. The relative standard deviations (%RSD) are more acceptable in the case of formoterol fumarate than fluticasone propionate, this latter being more cohesive and used at higher concentrations. Extending the concentration increased the %RSD but they remain acceptable except in the case of 3.5% of fluticasone propionate most probably because of the aggregation of the cohesive fluticasone propionate particles.

The blend with 3.5% fluticasone propionate presented greater variations and was different from the others with regard to the average content ($p < 0.05$). This was thought to be probably due to an excess of drug that cannot be fixed on the Lactohale 200 and formed agglomerates of pure drug responsible for the variations observed. This was confirmed by the visual observation of clusters in this blend, representing the aggregation of the cohesive fluticasone particles between them or with lactose fines. It was also confirmed by measurement of fluticasone size in the blends by laser diffraction after dissolving the lactose carrier in water (Le et al., in press).

3.2. Influence of drug concentration on adhesion characteristics

With regards to the evaluation of adhesion characteristics, Fig. 3 presents the percentage of formoterol fumarate remaining fixed to the carrier in relation to the functioning time of the Alpine air-jet sieve for the blends containing different percentage of formoterol fumarate. The formoterol fumarate was rapidly carried away by the airflow. The percentage of drug present after 5 s was an indicator of the percentage of drug that adhered to the carrier. Indeed, as drug particle size was much lower than 32 μm, if the drug particles were individualized in the blend and not adhered on the carrier, they would be carried away through the 32 μm sieve by aspiration.

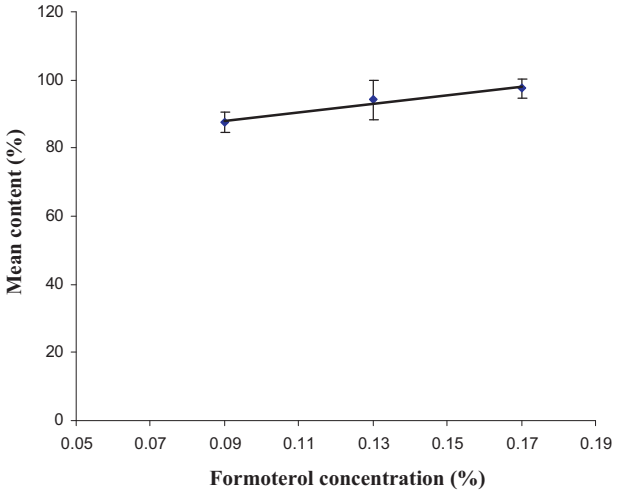

Fig. 2. Average content of formoterol fumarate in relation to the concentration.

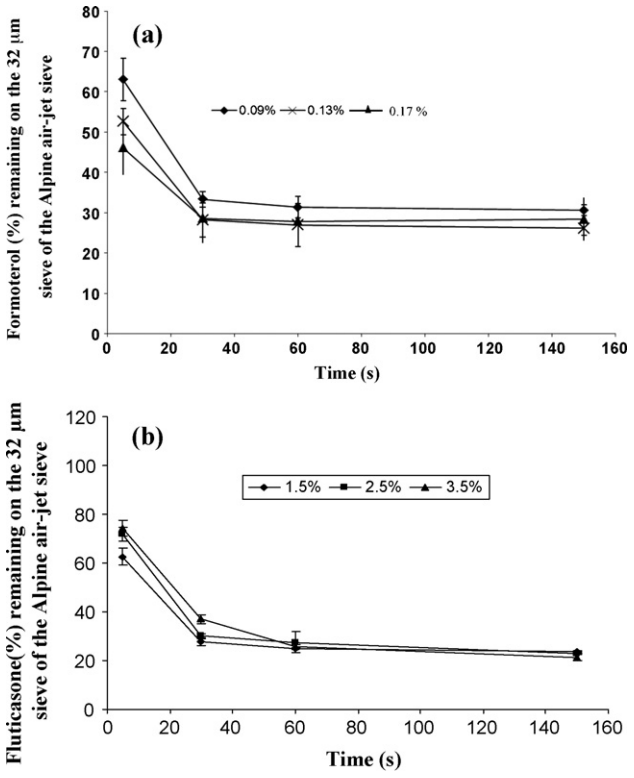

Fig. 3. Percentage of formoterol fumarate (a) and fluticasone propionate (b) remaining in the blend in relation to the functioning time of the air-jet sieve.

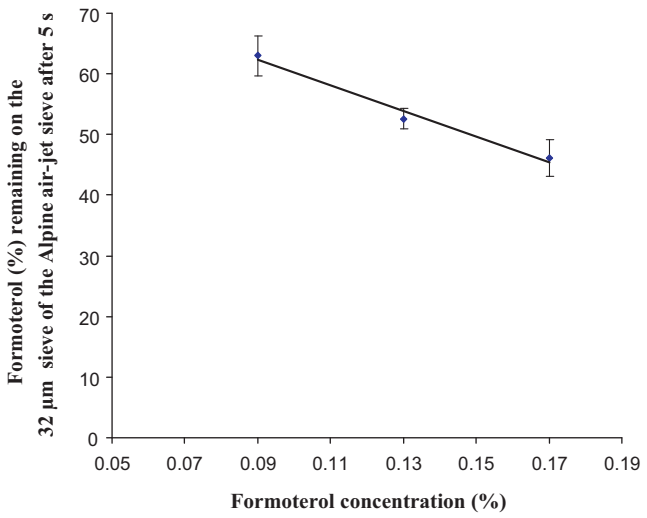

Fig. 4. Percentage of formoterol fumarate remaining on the 32 µm sieve of the air-jet sieve after 5 s in relation to the concentration of formoterol fumarate.

The percentage that remained adhered to the carrier after 5 s varied according to the formoterol concentration in the blend ($p < 0.001$). After 5 s, compared to the initial dose, about 63%, 53% and 46% of formoterol fumarate remained fixed on Lactohale 200 for the blends containing respectively 0.09%, 0.13% and 0.17% (w/w) of drug. The evolution with aspiration time showed drug detachment. If the aspiration time increased, the totality of the formoterol fumarate did not separate from the Lactohale 200 with variations according to the formoterol concentration ($p < 0.05$). After 150 s, an important percentage of formoterol fumarate (26–31%) kept adhering on the carrier (Fig. 3). The higher percentage (31%) remaining, observed with 0.09% (w/w) formoterol, indicated that the release was the lower for this concentration.

When the concentration in formoterol increased, a linear decrease ($R^2 = 0.983$) of the percentage of drug remaining on the sieve after 5 s was observed (Fig. 4). The behaviour of the lactose-drug blend during the assay can give an estimation of the drug capacity to separate from the carrier during inhalation from a Dry Powder Inhaler. Strong adhesion of the micronised drug during the assay pre-supposes difficult separation of the drug after patient inhalation or the need for greater inhalation airflow. For higher concentration of formoterol, it seemed that a lower percentage of drug adhered to the lactose.

Concerning the adhesion characteristics of fluticasone propionate on the Lactohale 200, little difference was observed with the fluticasone concentration (Fig. 3). Compared to the initial dose, the percentage of fluticasone propionate that remained on the 32 µm sieve after 5 s was about 65%, 72% and 74% for the blends containing respectively 1.5%, 2.5% and 3.5% (w/w) of drug. Here, when the concentration in fluticasone increased, a linear increase ($R^2 = 0.9027$) of the quantity of drug remaining on the sieve after 5 s was observed. This percentage was high whatever the fluticasone concentration. But, compared to the formoterol fumarate, the concentrations of fluticasone propionate used were much higher. So, the strong binding sites on the Lactohale 200 particles may have been covered and saturated by the fluticasone, even for the 1.5% (w/w) concentration, leading the drug particles to adhere less strongly to alternative binding sites and to be dislodged from the carrier particles during the mixing process (Sebti et al., 2007). This often leads to the aggregation of the drug particles and the formation of clusters. This was in agreement with the results of average content that showed greater variations with fluticasone propionate based blends, particularly at a concentration of 3.5% (w/w) probably because of the presence of many agglomerates.

During the evaluation of the adhesion characteristics, these clusters made of very cohesive drug particles were not dissociated after 5 s and were not able to pass through the 32 µm sieve and so remained on the sieve and were responsible for the high percentage of drug present at 5 s. This percentage decreased with aspiration time. In this case, it is supposed that the drag forces of the airstream overcame the adhesion forces of the particles and was able to break up the agglomerates.

3.3. Influence of drug concentration on aerodynamic behaviour

The aerodynamic behaviour of the Lactohale 200/drug blends was estimated with TSI making it possible to study the in vitro deposition profile of the drugs (Table 2).

The emitted doses obtained for the formoterol fumarate were between 72.90% and 81.58% and did not differ significantly with the concentration ($p > 0.05$). The fine particle fractions obtained were between 15% and 26.20% and differ significantly with the formoterol concentration ($p < 0.005$). The fine particle fractions are not very high and could be due to the low drug load in the blends (0.09–0.17%). Indeed, Steckel et al. (2004) obtained fine particle fractions of salbutamol higher than 39% in the case of high drug

Table 2
Emitted doses and fine particle fractions obtained from the different blends.

Drug in the blend (% w/w)	Percentage emitted	Fine particle fraction (%)
Formoterol fumarate		
0.09	73 (RSD: 4.8)	15 (RSD: 0.6)
0.13	81 (RSD: 10.8)	19 (RSD: 0.4)
0.17	81 (RSD: 3.7)	26 (RSD: 3.8)
Fluticasone propionate		
1.5	79 (RSD: 7.8)	34 (RSD: 2.7)
2.5	79 (RSD: 2.2)	40 (RSD: 0.5)
3.5	72 (RSD: 0.8)	36 (RSD: 2.7)

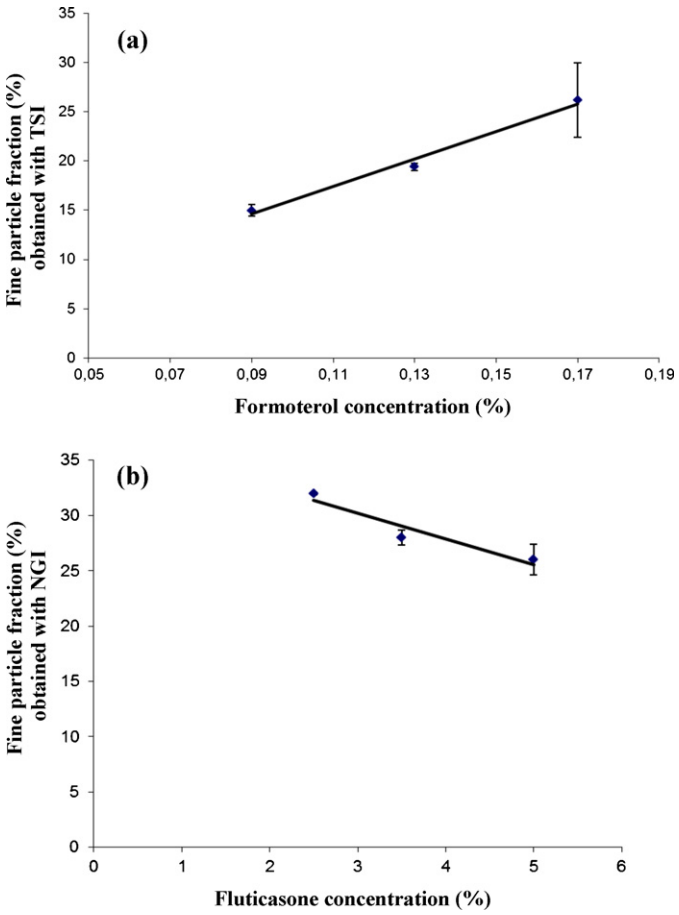

Fig. 5. Fine particle fraction in relation to the concentration of formoterol fumarate (a – obtained with TSI) and of fluticasone propionate (b – obtained with NGI).

load (2.8% w/w) and ranged from 12% to 27% for low drug load (0.25% w/w). The high fine particle fractions were attributed to the drug overage that led to adhesion of drug particles to the crystal surfaces with the highest surface energy with a huge proportion of drug being adhered to the low energy sites of the crystals.

Fig. 5 presents the fine particle fraction in relation to the concentration of formoterol fumarate. The fine particle fraction increased with the drug concentration. This was in agreement with the adhesion test that showed lower adhesion of formoterol fumarate when the concentration increased.

A linear relationship ($R^2 = 0.985$) between the fine particle fraction and the formoterol fumarate concentration was observed. It may be related to the fact that the detachment of formoterol fumarate from the Lactohale 200 was lower when the concentration was low in the mixture since the drug particles initially adhered to the high energy adhesion sites on the carrier. An increase in the drug concentration led to the saturation of the carrier sites with the strongest binding forces. At higher drug concentrations, more drug particles may be adhering to sites with less strong binding affinities on the surface of carriers, thus enabling greater drug detachment. Another possibility is the formation of aggregates between the drug in excess and the fine particles of lactose contained in the Lactohale 200 (in our case, 10% particles below 7.69 μm and 19% under 32 μm). In this theory, also called redistribution theory, aggregates of drug and lactose fine particles termed multiplets are formed which increases the detachment forces during inhalation (Louey and Stewart, 2002; Lucas et al., 1998). In our case, the fine particle multiplets theory seems to be predominant and the agglomeration hypothesis was shown after comparison of the performances of blends with sieved and un-sieved Lactohale 200 (Le et al., 2012).

The emitted doses obtained for the fluticasone propionate were between 71.60% and 79.52% and did not differ significantly with the concentration ($p > 0.05$).

The fine particle fractions obtained were between 33% and 40% and differ significantly with the fluticasone concentration ($p < 0.001$). First, the fine particle fraction increased with the fluticasone concentration and then decreased with a further increase of the fluticasone concentration. These results are in agreement with those obtained by APTAR PHARMA and their new device Prohaler®. For concentrations of 2.5, 3.5 and 5% (w/w) of fluticasone in lactose, the emitted fractions of fluticasone and fine particle fractions were 81%, 83%, 79% and 32%, 28%, 26% respectively. A linear relationship ($R^2 = 0.91$) between the fine particle fraction and the fluticasone concentration was observed (Fig. 5). Beyond 2.5% (w/w), increasing the fluticasone concentration decreases significantly the fine particle fraction. Even if the 1.5% concentration was not tested with the Prohaler, it can be supposed that the behaviour would be the same as for the Inhalator. Indeed, we made many blends whose behaviour in the Prohaler and in the Inhalator was compared and the same tendency was observed.

The increase in drug loading is in favour of higher fine particle fractions but until an optimum beyond which the excess of drug

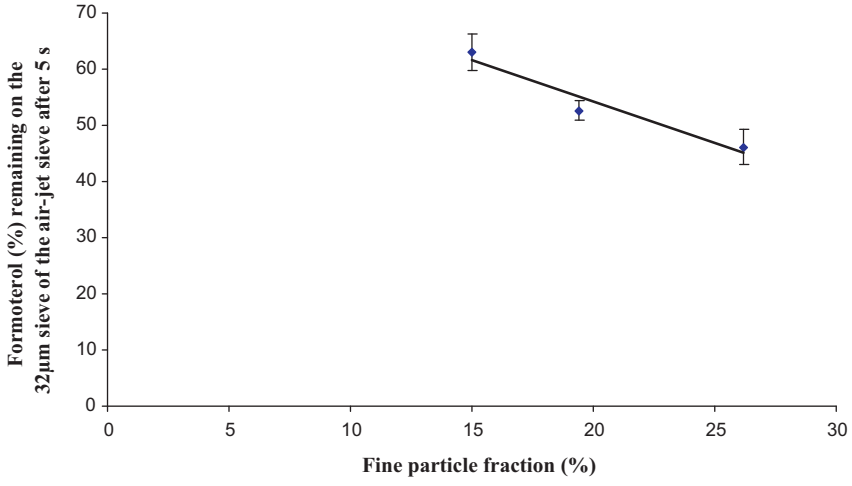

Fig. 6. Relation between fine particle fraction and percentage of formoterol fumarate remaining on the 32 μm sieve of the air-jet sieve after 5 s.

forms agglomerates of the cohesive drug particles leading to a lack of homogeneity and uncertain delivery of the drug. This is in agreement with the observations of Podczeck (1998) who reported that if detached drug agglomerates are strong enough to withstand the forces needed to break them up, the fine particle fraction will be unsatisfactory (De Boer et al., 2005).

3.4. Correlation between adhesion characteristics and inertial impaction

The results of drug separation from the carrier by sieving with the Alpine air-jet sieve were compared with those obtained from in vitro deposition studies with the Twin Stage Impinger for the formoterol fumarate. Fig. 6 represents the relationship between fine particle fraction and the percentage of formoterol fumarate remaining on the 32 μm sieve of the air-jet sieve after 5 s. A linear relationship was noted between them with a coefficient R^2 of 0.937 which indicates a good correlation between these two parameters. These results concern the first 5 s of aspiration which could be compared to the inhalation time of a patient when using a dry powder inhaler. The same tendency was observed for fluticasone propionate.

The method we proposed using Alpine air-jet sieve is simple, easy to perform, making it possible to forecast drug detachment from the carrier and to predict the aerodynamic behaviour of the drug. This method of adhesion evaluation considered the whole blend as it is used in dry powder inhalers which was different to techniques like Atomic Force Microscopy that concerns only one particle and not the overall blend (Louey et al., 2001).

4. Conclusion

Drug concentration in dry powder inhalers influences adhesion, content uniformity and in vitro deposition of the drug. This influence depends on the drug considered and its range of concentration.

The formoterol fumarate concentration in adhesive mixtures influences its adhesion with Lactohale 200 and its detachment from this carrier. For the higher concentration of formoterol fumarate, a lower quantity of drug adheres to the lactose. The fine particle fraction increases with the formoterol fumarate concentration in the range of concentration tested. There is a linear relationship between the formoterol fumarate concentration and the fine particle fraction. Adhesion testing using air-jet sieving assays makes it possible to predict drug separation from the lactose carrier faster and more simply than from assays with an impactor.

In the case of fluticasone propionate, the influence of the concentration is different, but the concentrations tested are higher. First, the fine particle fraction increases with the concentration and then decreases with a further increase of the fluticasone concentration. If the drug concentration is too high, there is an excess of drug that can not be fixed on the carrier and forms agglomerates of pure drug responsible for a lack of homogeneity and of a decrease in fine particle fraction.

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