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Research paper

Impact of feed counterion addition and cyclone type on aerodynamic behavior of alginic-atenolol microparticles produced by spray drying

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ABSTRACT

The inhalatory route has emerged as an interesting non-invasive alternative for drug delivery. This allows both pulmonary (local) and systemic treatments (via alveolar absorption). Further advantages in terms of stability, dose and patient preference have often lead researchers to focus on dry powder inhaler delivery systems. Atenolol is an antihypertensive drug with low oral bioavailability and gastrointestinal side effects. Because atenolol possesses adequate permeation across human epithelial membranes, it has been proposed as a good candidate for inhalatory administration. In a previous work, atenolol was combined with alginic acid (AA) and microparticles were developed using spray-drying (SD) technology. Different AA/atenolol ratios, total feed solid content and operative variables were previously explored. In order to improve particle quality for inhalatory administration and the SD yield, in this work the AA acid groups not neutralized by atenolol were kept either free or neutralized to pH ~ 7 and two different SD cyclones were used. Particle morphology, flow properties, moisture uptake and *in vitro* aerosolization behavior at different pressure drops were studied. When the AA acid groups were neutralized, particle size decreased as a consequence of the lower feed viscosity. The SD yield and *in vitro* particle deposition significantly increased when a high performance cyclone was employed, and even when lactose carrier particles were not used. Although the *in vitro* particle deposition decreased when the storage relative humidity increased, the developed SD powders showed adequate characteristics to be administered by inhalatory route up to storage relative humidities of about 60%.

1. Introduction

Inhalatory route constitutes an alternative to other administration routes to deliver drugs for local or systemic treatments in a noninvasive way. Both pulmonary and nasal administration may improve drug bioavailability, because the hepatic first-pass metabolism is avoided and an extensive surface area and an epithelial layer highly vascularized are available for absorption. Drugs with low oral bioavailability and gastrointestinal side effects are good candidates for inhalatory administration [1]. Among others, hormones, adrenergic beta-blockers, cardiovascular drugs, non-steroidal antiinflammatory drugs, peptides, and proteins have been proposed to be administered by the inhalatory route [1]. To develop effective respiratory drug delivery systems, for a diverse range of therapeutics, different formulation and processing strategies have been evaluated [2].

Among the different inhalation device systems, dry powder inhalers (DPIs), have begun to be the preferred platform for new inhaled products and also generally in preclinical reports. This preference has been attributed to the well described advantages in terms of stability, dose and efficiency. However, to deliver active pharmaceutical ingredients (APIs) to the action or absorption sites in the inhalatory tract, it is required an appropriate design of the particulate system.

Inhalable particles usually exhibit poor flow properties and tendency to agglomerate. These properties are relevant in the dosing process during industrial DPIs production and the aerosolization step when the dry powder is administered to patients [3]. For this reason, the drug particles are usually mixed with larger carrier

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particles. Among other excipients, lactose and mannitol have been used. In particular, lactose is commonly used because of its favorable properties: non-toxicity, physicochemical stability and biocompatibility [4].

Particle deposition has to be studied to properly design particles for DPIs. The powder capability to reach the respiratory membrane can be estimated by assessing the aerodynamic particle size distribution. To this end, official compendia recommend the use of multi-stage cascade impactors or multi-stage cascade impingers [5]. The Next Generation Pharmaceutical Impactor (NGI) is widely used. It has seven separation stages and a final micro orifice collector (MOC) [6]. The recovery of the drug mass deposited on each stage allows its quantification within specific ranges of aerodynamic diameters, and therefore estimates the powder's ability to reach the lung respiratory region. Aerodynamic diameters lower than 3 μm determined in impactors demonstrated close equivalence to in vivo lung deposition (assayed by gamma scintigraphy) [7]. One important factor that affects drug deposition in the NGI assay is ambient moisture as at high relative humidity capillary forces increases and aerosolization performance decreased because it is more difficult to break the agglomerates into individual particles [8,9].

The inhalatory route to administer cardiovascular drugs is an interesting alternative since it overcomes difficulties that face conventional routes. Atenolol is an antihypertensive drug with low oral bioavailability (~50%) due to its poor intestinal absorption [10]. Atenolol is formulated in tablets (chronic treatment of the illness) or parenteral solutions (early intervention treatment of myocardial infarction). Recently this active ingredient has been proposed as an interesting therapeutic option for pulmonary hypertension [11]. When orally administered, it can cause undesirable central nervous system effects such as lethargy and tiredness, which limit patient adherence. As stated by Gostick et al., tiredness is dose related, becoming more important when atenolol dose is higher than 100 mg per day [12]. Also, gastrointestinal side effects such as nausea, ischemic colitis and diarrhea among others have also been reported with an incidence of 8.8% [13]. To overcome these side effects and increase systemic bioavailability, transdermal drug delivery systems of this drug have been proposed. However, penetration enhancers or microneedles are required in order to obtain an adequate atenolol transdermal delivery [10,14]. Because atenolol possesses adequate permeation in human airway membranes [15] and lower doses should be necessary compared to the oral route, it has been proposed as a good candidate for inhalatory administration in order to increase its bioavailability and avoid the gastrointestinal side effects. In this sense, Rabinowitz and Zaffaroni developed an evaporation/condensation aerosol inhalation formulation based on this drug [16].

Atenolol is around 20 times less potent at blocking β-2-receptors than blocking β-1-receptors. Although it is recommended to use this drug cautiously in people who suffer from asthma [17], two meta-analyses published by Selpeter et al. indicated that atenolol at doses between 50 and 200 mg in patients who suffer from obstructive lung diseases did not demonstrate respiratory side effects [18,19]. Furthermore De Plaen et al. demonstrated that even at 600 mg intravenous dose, this drug did not demonstrate β-2-blockade [20]. Rabinowitz and Zaffaroni proposed inhalatory atenolol doses between 0.1 mg and 20 mg, which should not display respiratory adverse effects [16]. Thus, the atenolol recommended dose is well below the ones that have not demonstrated respiratory side effects.

As a new formulation approach, in a previous work atenolol was combined with alginic acid (AA) and microparticles were obtained by spray drying (SD). By manipulating the SD feed composition (aqueous dispersions with different atenolol/AA ratios) and operating variables, this process was able to produce suitable materials for inhalatory delivery [21]. It was demonstrated that the formulation components interacted ionically leading to a new chemical entity. The ionic interaction between both components was a valuable resource to modify the physicochemical properties of the raw materials. The proposed new materials exhibited flexibility to load different drug contents in amorphous state and adequate estimated mean aerodynamic diameters for inhalatory administration [21].

To the best of our knowledge, the aerodynamic performance of particles based on polyelectrolyte-drug complexes has not been previously reported. As changes in the spray drying feed composition affect product' quality at molecular and particle levels, there is a need for studying these systems in order to find relationships between process parameters, feed composition and powder' attributes relevant for this type of inhalable systems.

In this context, the aim of this work was to evaluate the aerodynamic behavior of particles based on AA-atenolol complexes. To this end and based on previous results [21], improved powders were obtained by varying the pH of the SD feed dispersions and using different collection cyclones (standard and high performance ones). The impact of the KOH addition to adjust the feed pH on the product quality and process performance was assessed by evaluating particle properties (atenolol load efficiency, moisture content, morphology, particle size distribution and density) and the SD yield. In addition, the influence of particle composition, pressure drop and powder storage relative humidity on *in vitro* particle aerosolization was evaluated by means of a NGI equipment. Fine particle and respirable fractions lower than 3 μm were determined as particles intended for systemic delivery need to display this small aerodynamic diameter in order to reach the alveolar region and thus being absorbed.

2. Materials and methods

2.1. Materials

Alginic acid (AA) from Brown Algae (analytical grade, Sigma, Saint Louis, United States), atenolol (pharmaceutical grade, Parafarm, Saporiti, Buenos Aires, Argentina), lactose monohydrate −140 +270 ASTM Mesh (pharmaceutical grade, Parafarm, Saporiti, Buenos Aires, Argentina), potassium hydroxide (analytical grade, Cicarelli, Santa Fé, Argentina), size 3 gelatine capsules (pharmaceutical grade, Parafarm, Saporiti, Buenos Aires, Argentina), glycerin (Anedra, Buenos Aires, Argentina) and distilled water were used.

2.2. Methods

2.2.1. Dispersion preparation and characterization

Dispersions for spray drying were prepared according to the compositions detailed in Table 1 and following the methodology previously explained [21]. The atenolol/AA ratio was fixed in order to obtain a 75% of neutralization of the available acidic groups of AA (4.55×10^{-3} equivalents per AA gram [21]). The AA concentration (a viscosity increasing agent) in the dispersion was 1% w/v. The pH of the SD feed dispersion was adjusted close to 7 by adding KOH (0.09567 N) to some selected dispersions (Sample II, Table 1). Sample I (Table 1) corresponded to non-pH-adjusted dispersions. The following dispersion physicochemical properties were measured in triplicate: pH: by using a pH meter Orion 410A (Cole Parmer, Vermon Hills, United States); *Kinematic viscosity* (*at* 25 °C): employing a capillary Cannon–Fenske Routine-type viscometer, tube size 100 (Cannon Instrument Company, State College, United States).

Table 1
Composition and properties of the dispersions feed to the spray dryer (in 200 mL).

Sample	Alginic acid (g)	Atenolol(g)	KOH (g)	pH	Viscosity (mm^2/seg)
I	2.00	1.82	–	4.13 ± 0.04	4.65
II	2.00	1.82	0.13	6.53 ± 0.03	3.65

Sample I: obtained by mixing AT and AA in a ratio to neutralize the 75% of the AA available acidic groups. Sample II: obtained by mixing AT and AA in a ratio to neutralize the 75% of the AA available acidic groups and adjusting the final pH to almost 7 by adding KOH.

2.2.2. Spray drying

Dispersions were atomized under constant magnetic stirring in a negative pressure laboratory scale SD equipment (Mini Spray Dryer B-290, BÜCHI, Flawil, Switzerland). A two-fluid nozzle with a cap-orifice diameter of 0.5 mm was used. Operating conditions were selected accordingly to a previous work [21]: air inlet temperature (co-current): 140 °C, liquid feed flow rate: 20% (6 mL/min), atomization air flow rate: 742 L/h and drying air flow rate: 35 m^3/h. Two different cyclones were used in order to collect the powders: a standard cyclone (SC) and a high performance cyclone (HPC). The collected powder was weighed, packed in sealed amber bottles and stored in a desiccator for further characterization. For comparison purposes, an atenolol aqueous solution was spray dried [21]. The process yield was calculated as the ratio of the weight of product collected after spray drying to the initial amount of solids used to prepare the aqueous dispersions.

2.2.3. Powder characterization

Moisture content: it was determined immediately after the spray-drying process in a halogen moisture analyzer (MB45, Ohaus, Pine Brook, United States). About 500 mg of powder was heated up to 105 °C until the weight change was less than 1 mg in sixty seconds.

Drug loading efficiency: atenolol mass concentration of the obtained products was determined by UV-spectrophotometry at 274.6 nm by dissolving the powders in distilled water following the methodology explained by Ceschan et al. [21].

Particle size distribution was measured by laser diffraction using the dry powder method (LA 950V2, Horiba, Kyoto, Japan). The SD powders were dispersed in lactose to improve the sample flow from the feed hopper to the measuring cell as previously explained [21]. Size is reported as mean volumetric diameter (D_{43}) and distribution width is informed as *span*. *Span* index is calculated as shown in Eq. (1).

$$Span = \frac{(D90 - D10)}{D50} \quad (1)$$

where D_{90}, D_{50} and D_{10} are the diameters where the 90%, 50% and 10% of the population lie below each value, respectively. A distribution can be considered relatively narrow if the *span* value is less than 2 [22].

Particle morphology was evaluated through Scanning Electron Microscopy (SEM). Samples were metalized with gold (~300 Å coating thickness, Sputter Coater 91000, PELCO, TellPella, Canada) and they were observed and photographed using and EVO 40-XVP, LEO scanning electron microscope (Oberchoken, Germany).

Skeletal density of the SD products was determined by nitrogen adsorption (Nova 1200e, Quantachrome Instruments, Florida, United States). A sample of 1 g was placed in a precalibrated cell and its volume was determined by the intrusion of nitrogen as explained by Ceschan et al. [21].

Bulk (D_{bulk}) and tapped (D_{tap}) densities were determined by using a 10 cm^3 graduated cylinder as explained in the USP Pharmacopoeia [23]. All determinations were made in triplicate.

Carr Index (CI) was evaluated using the bulk and tapped densities as follows:

$$CI = (D_{tap} - D_{bulk})100/D_{tap} \quad (2)$$

Results were interpreted according to the USP Pharmacopoeia [23].

Additionally, and with the aim to evaluate the possible powder flow improvement when lactose is used as a carrier, SD samples were mixed with lactose in a 1:3 microparticles:lactose ratio and bulk and tapped densities were re-assayed.

2.2.4. Blends of SD powders with lactose carrier

SD powders and lactose, used as a carrier, were mixed in a 1:3 ratio by geometric dilution. The mixtures were blended using a Turbula® orbital mixer (Glen Mills, Maywood, United States) at 46 RPM for 20 min. Content uniformity was evaluated in 5 random samples (25 mg each) by UV spectrophotometry at 274.6 nm. Blends were stored in a dessicator.

2.2.5. In vitro particle deposition

The *in vitro* aerosolization performance of the SD powders was evaluated in a Next Generation Impactor (Copley Scientific, Nottingham, UK) equipped with an induction port (IP) and a pre-separator (PS). The inhaler was connected via a mouthpiece adapter (MA) to the IP. The NGI is constituted by seven-stage inertial impactor that separates the powder into different ranges of aerodynamic diameters and, as a final stage, by a micro-orifice collector (MOC) [24].

Size 3 gelatin capsules were filled with 25 ± 0.50 mg of SD powders or with the appropriate amount of SD powder:lactose carrier mixture 1:3 containing 25 mg of SD powder. Powders were dispersed through an RS01 high resistance inhaler (Plastiape, Milano, Italy) into the NGI. Two pressure drops (ΔP) were assayed: 2 and 4 kPa with or without using the pre-separator NGI component, respectively. The air flow rates for the pressure drops 2 and for 4 kPa were 42.5 and 58.8 L/min, respectively. Although USP specifies as a standard test condition 4 kPa, increasing attention has been paid to evaluate the performance of formulations at lower flow rates (i.e., lower pressure drops), in order to assess whether patients with weaker inhalation profiles can still reach inhalatory therapeutic doses [25,26]. For this reason the formulations have been also tested at 2 kPa. The NGI was run enough time to allow 4 L of air through the equipment. For both flow rates, the aerodynamic cutoff diameters for each stage of the impactor were calculated following the guidelines given by Marple et al., USP and Ph. Eur [23,27,28].

To avoid particle re-entrainment/bouncing, the NGI stages were coated with glycerol. The pre-separator (PS) was loaded with 15 mL of water in order to recover the deposited drug. The drug deposited in all the NGI components was collected by rinsing each part with water. Drug content was assessed using a UV-spectrophotometer at 274.6 nm.

The Emitted Fraction (EF), Fine Particle Fraction (FPF), Respirable Fraction (RF), Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) were determined as follows [29]: *EF:* represents the drug percentage of total drug loaded in the capsule that is effectively released from the capsule and the inhaler; *FPF:* is the percentage of cumulative drug mass with aerodynamic diameters lower than 3 μm with respect

to the total drug mass recovered from the MA, PS, IP, NGI 1–7 stages and MOC; *RF:* accounts for the cumulative percentage of drug mass with aerodynamic diameters lower than 3-μm with respect to the total drug mass recovered from the capsule, inhaler, MA, PS, IP, NGI 1–7 stages and MOC.

The mass median aerodynamic diameter (MMAD) was calculated from a drug mass cumulative distribution (built considering the drug mass collected in NGI-1–7 stages and MOC) and is defined as the diameter at which 50% of the drug is collected in larger particles and the remaining 50% is collected within smaller particles. For systemic inhalatory administration, MMAD values should lie between 0.5 and 3 μm [30].

The geometric standard deviation (GSD), that represents the spread of an aerodynamic particle size distribution, was calculated as $(D_{84}/D_{16})^{1/2}$, where D_{84} and D_{16} represent the diameters at which 84% and 16% of the drug mass are recovered from the NGI 1–7 stages and MOC, respectively. Particle aerodynamic size distribution is considered narrow if GSD is lower than 3 [31].

2.2.6. Moisture absorption, aerodynamic and morphological characterization after storage

Moisture uptake of products was evaluated at 40, 60 and 75% relative humidity (RH) at room temperature. These RHs were provided by mixtures of glycerol and water according to the ASTM D5030-97 norm [32]. 100 mg of samples I and II obtained using the HPC was stored for 24 h in hermetic recipients. Also, a blend of SD microparticles and lactose (1:3 microparticles:lactose ratio) was stored under the same conditions than the SD microparticles. The samples were reweighed and the weight increase was associated with the water adsorption. Immediately after that, particles were assayed again in the NGI equipment in order to evaluate the influence of the storage at different RHs on the *in vitro* particle deposition performance. Also, samples were observed in a LEO scanning electron microscope (Oberchoken, Germany), under variable pressure (10 kV, 70 Pa chamber pressure, 40 pA beam current) [33]. This technique offers the possibility to characterize the morphology of samples with a certain degree of humidity, without preparation or special treatments [34].

2.2.7. Statistical analysis

The significant differences between the process yield, samples densities and *in vitro* particle deposition behavior were determined by means of one-way ANOVA, followed by the Least Significant Difference (LSD) post hoc multiple comparison method. Statistical significance was established through the *p*-value: values lower than 0.05 were considered statistically significant. Before the analysis, homoscedasticity and normality ANOVA's assumptions were checked by the Levene test and Standard Kurtosis values, respectively [35,36].

3. Results and discussion

3.1. Dispersion characterization

Dispersion properties (pH and viscosity) were characterized before being fed in the spray dryer (Table 1). The pH of sample II was adjusted to values close to 7, while the pH of sample I was unchanged. KOH was added to the formulations to increase the pH from 4.1 (sample I) to approximately 6.5 (sample II). Although the pH of sample I is low, Aris et al. indicated that 9 volunteers exposed to nebulized solutions of pH 4 (MMAD: 6.1 ± 1.5 μm) experimented only moderate throat irritation and only one subject enrolled in the study demonstrated moderate breath shortness and production of sputum [37]. In fact, the lung mucus plays a defense role against acidic components [38]. In order to prevent irritation, the use of KOH to neutralize the formulations allows improving biocompatibility of the microparticles [39,40].

As can also be seen in Table 1, kinematic viscosities of the formulations decreased (by about 20%) when pH was increased. Viscosity is known to be an important feed property that affects droplet and particle size and morphology following spray drying. When the dispersion viscosity decreases, the atomized droplets are smaller and then the drying performance is enhanced [41]. This viscosity decreases, when the neutralization of the AA acidic groups increased, as is consistent with previous reports [21] and could be related to the charge density and chain conformation of the polyelectrolyte macromolecule [42]. The viscosity effect on particle size will be discussed below.

3.2. Spray-drying

Spray drying has been widely used to produce particles for inhalation by controlling the feed formulation and operating parameters, and by doing this, the product quality can be modified to some extent [3]. In a previous work, microparticles for atenolol inhalatory administration were obtained using an atomization air flow rate of 600 L/h and a standard cyclone [21]. Although estimated aerodynamic particle diameters obtained under these conditions were adequate for microparticle inhalatory administration, an improvement of particle quality was proposed by increasing the atomization air flow rate to 742-L/h. This increase led to smaller particles; however, the yield decreased by 32% [21].

In this work, the atomization air flow rate was fixed at 742 L/h and two different cyclones (SC and HPC) were used. The dispersions prepared according Table 1 were fed to the spray dryer. Table 2 shows outlet air temperatures (T_{out}) as well as drying process yield.

For all the experiments, T_{out} resulted to be well below than the degradation temperatures of atenolol and AA [43,44]. The use of the HPC led to lower air outlet temperatures and higher residual moistures than when the SC was used. The same trend was observed by Maury et al. [45].

As can be seen in Table 2, the yield increased markedly when a high performance cyclone (HPC) was used. In fact, samples I and II exhibited a yield improvement of 85% and 64%, respectively, when the SC was replaced by the HPC. As demonstrated, the cyclone type had a great impact on the SD yield. This could be related to the capability of HPC to collect smaller particles [46]. This issue will be further addressed below in Section 3.3.2.

Regarding the change in feed composition, for both cyclone types, the addition of KOH did not modify the yield value significantly (*p*-value > 0.05). Consequently, the observed changes in viscosity (due to the different pH feed values) did not affect the product recovery.

3.3. Product characterization

3.3.1. Moisture content and drug load efficiency

The residual moisture content for all the products is shown in Table 2. As can be seen, all samples exhibited moisture contents lower than 5%, indicating that the drying process under the selected SD variables was efficient [47].

The drug load efficiency is close to the expected composition (0.48 and 0.46 $g_{Atenolol}/g_{powder}$ for samples I and II, respectively) and with mean errors lower than 11%. The high capacity of the microparticles to carry atenolol is also a valuable attribute for the delivery of this drug to the respiratory tract.

Table 2
Outlet air temperature, spray drying yield and powder properties (moisture content, drug load efficiency, mean volumetric diameter and span).

Sample	T_{out} (°C)	Yield (%)	Moisture content (%)	Drug load efficiency ($g_{Atenolol}/g_{powder}$)	D_{43} (μm)	Span
I SC	80.2 ± 1.8	45.84 ± 2.64	3.95 ± 0.14	0.54 ± 0.01	5.15 ± 0.11	1.11 ± 0.12
II SC	85.0 ± 1.4	53.48 ± 1.11	2.97 ± 1.10	0.49 ± 0.01	3.95 ± 0.21	1.71 ± 0.04
I HPC	78.5 ± 2.5	85.18 ± 0.52	4.69 ± 0.78	0.53 ± 0.01	3.76 ± 0.35	1.86 ± 0.14
II HPC	77.8 ± 2.3	87.80 ± 1.34	3.99 ± 0.76	0.48 ± 0.01	3.40 ± 0.10	1.69 ± 0.07

T_{out}: Outlet air temperature. Experiments were done in triplicate. D_{43}: mean volumetric diameter. Alginic-atenolol (Sample I) and alginic-atenolol-potassium (Sample II) microparticles obtained by spray drying using a standard (SC) or a high performance cyclone (HPC).

3.3.2. Morphology and particle size distribution

Different feed formulation properties and operating conditions allow producing particles with different morphology [48]. SD microparticle morphologies were studied by scanning electronic microscopy (SEM) and micrographs are shown in Fig. 1. Besides, parameters related to the particle size distribution of the SD powders are shown in Table 2.

Fig. 1 shows the SD microparticles obtained for the formulations I and II and using different cyclone types. As it can be seen, all SD materials presented wrinkled particles. The addition of KOH did not significantly affect particle morphology as it can be observed in Fig. 1a and c. Corrugated particle surfaces have been related to better aerosolization performance and lower capsule retention in *in vitro* particle deposition assays. This behavior is attributed to the fact that this type of particles provides less surface contact points and then lower tendency for agglomeration or stickiness to the inhaler surface [3].

Regarding particle size distribution, D_{43} was between 3.40 and 5.15 μm. Table 2 shows that these values depend on both the SD feed composition and the cyclones used to collect the dried microparticles. Regarding the effects of composition, higher viscosities (Sample I) led to larger particles because bigger droplets are produced by atomization than the ones generated by the lower viscosity pH-adjusted dispersions [49]. This effect could be observed even when the solid content was higher (as KOH was added in sample II). The effect of the cyclones used to collect the particles is related to its geometry; standard cyclones cannot collect particles smaller than 2 μm [45], while the HPCs have the capability to capture particles of 1 μm [50].

Sample II HPC exhibited the smallest mean volume particle size as a result of combination of the lower SD feed viscosity compared with that of Sample I and the use of the HPC. The particle size distributions of the obtained powders (see mean volume diameter and span; Table 2) indicate that the selected SD operating conditions were adequate to obtain narrow distributions of small particles together with high process yields.

3.3.3. Powder densities and flow properties

Different densities and the Carr Index of the SD powders are shown in Table 3. Particularly, the skeletal densities were between 0.92 and 1.10 g/cm^3. This property for Samples II were around 15% higher than those measured for Samples I, being statistically significant (p-value < 0.05). This behavior has been previously reported and could be related to the capability of solid components of less viscous droplets to migrate to the center during the evaporation when are being drying leading to denser particles [51,52].

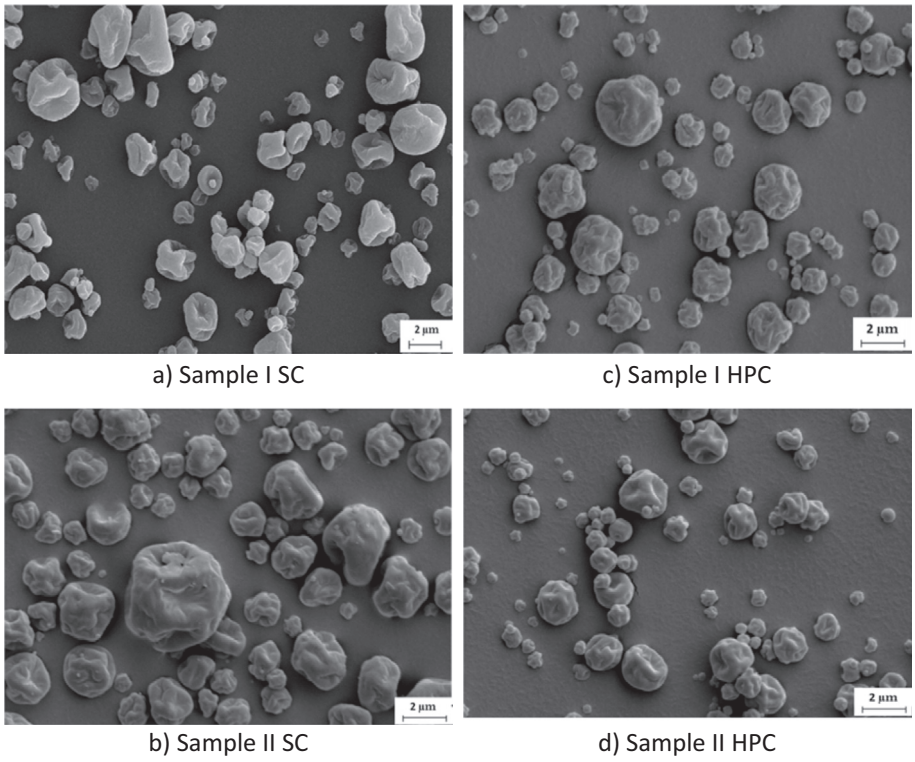

Fig. 1. Scanning electron microscopy micrographs of alginic-atenolol (Sample I) and alginic-atenolol-potassium (Sample II) microparticles obtained by spray drying using a standard (SC) or a high performance cyclone (HPC). Magnification: 15,000×.

Table 3
Powder densities and Carr index.

Samples	Skeletal density (g/cm^3)	D_{bulk} (g/cm^3)	D_{tap} (g/cm^3)	CI (spray-dried powders)	CI (1:3, spray-dried powder: lactose ratio)
I SC	0.93 ± 0.06	0.22 ± 0.01	0.33 ± 0.01	34.79 ± 2.02	29.87 ± 1.23
II SC	1.07 ± 0.05	0.26 ± 0.02	0.39 ± 0.04	32.36 ± 1.10	24.72 ± 2.09
I HPC	0.92 ± 0.09	0.30 ± 0.02	0.45 ± 0.01	32.58 ± 2.72	26.83 ± 1.89
II HPC	1.06 ± 0.07	0.29 ± 0.03	0.45 ± 0.04	36.37 ± 1.76	24.07 ± 1.75

Experiments were done in triplicate. D_{bulk}: bulk density; D_{tap}: tapped density; CI: Carr Index. Alginic-atenolol (Sample I) and alginic-atenolol-potassium (Sample II) microparticles obtained by spray drying using a standard (SC) or a high performance cyclone (HPC).

Bulk density was between 0.22 and 0.30 mg/mL while tapped density was between 0.33 and 0.45 mg/mL. Both samples obtained using the HPC exhibited higher tapped density because small particles have the ability to occupy the voids left for the bigger particles [53]. According to the USP classification, the CI values of all samples indicated that the powders presented very poor flow properties [23]. This result was expected considering the small volumetric median particle diameters found for all the powders. Inhalatory particles are, in general, cohesive powders and tend to agglomerate as van der Waal attractive forces dominate the particle-particle interactions for fine powders with median diameters smaller than 30 μm [54]. It has been also reported that flow can be improved using lactose-carrier particles [29]. For this reason, AA-atenolol samples were mixed with lactose in a 1:3 SD microparticles:lactose ratio. Bulk and tapped densities were re-assayed in order to calculate the blend's CIs. The results, also shown in Table 3, indicate that effectively, the addition of lactose has the capability to increase the powder flowability. In fact, the CI values of the powder's blends indicate that the flowability is acceptable for Samples II and poor for Samples I [23].

Flow properties are relevant during dosing in order to achieve accurately the effective drug dose. For volumetric filling, average flow properties are necessary for DPI inhalers [55,56] and carrier particles could be needed [57]. In fact, SD powder flowability of the SD microparticles carrying atenolol improved when lactose was added. These blends could be adequate for standard dosing equipment as better handling is achieved. However, considering that some formulations are postulated as carrier-free developments, increasing interest has been paid to develop adaptations on capsule filling machines [58]. Using them, cohesive inhalatory powders with bulk density between 0.10 and 0.45 g/mL could be dosed [55,59]. According to this, the SD powders obtained would be adequately dosed as carrier-free systems.

3.4. Aerosolization behavior

3.4.1. In vitro test deposition

Table 4 shows the aerodynamic properties of the SD microparticles assayed in the NGI. The aim of this study was to determine the *in vitro* deposition of the different samples, as carrier-free formulations or blended with lactose coarse particles, at different pressure drops.

For carrier-free particles at a pressure drop of 4 kPa, all the samples exhibited high emitted fractions (EF values between 90.9 and 94.3%) regardless of the SD feed composition and the cyclone type. FPF and RF were between 14.5–29.1% and 13.3–26.5%, respectively. These values indicate that the formulations were adequate for atenolol pulmonary administration. In fact, commercially available DPIs have a FPF within the 10–35% range [30]. It is important to note that the use of the HPC improved the FPF and RF values by 90% and 46% for Samples I and II, respectively, with respect to the values obtained when the SC was employed. These improvements were statistically significant (p-values < 0.05) and were related to the capability of the high performance cyclone to collect small particles (see mean volume diameters, Table 2).

Considering that samples I and II HPC exhibited the best deposition performance at 4 kPa, both formulations were tested by setting a lower pressure drop (2 kPa). As it can be seen in Table 4, for both pressure drops and for carrier-free powders, adequate *in vitro* deposition is observed. For samples I and II HPC, a slightly EF decrease was observed when the pressure drop changed from 4 to 2 kPa. The decreases for the FPF and RF, caused by the pressure drop decrease, were not statistically significant for Sample I but led to statistically significant differences in the FPF and RF values for Sample II. Nevertheless, the results obtained were still adequate for lung administration. Although *in vitro* particle deposition for Sample II is dependent on flow rate, this formulation is adequate for atenolol inhalatory administration even at low flow rates. This fact is particularly relevant for patients with reduced inhalatory capacity [57]. It should be also taken into account that oral atenolol exhibits significant bioavailability variations within patients at therapeutically effective doses [60–63].

To evaluate the effect of lactose carrier particles on the microparticle aerosolization, blends of SD microparticles:lactose at the ratio 1:3, based on Samples I and II HPC, were assayed in the NGI and the results for different pressure drops are also shown in Table 4. As it can be seen, slight EF improvements were observed for blends with lactose. Although it has been reported that this car-

Table 4
NGI Deposition parameters (EF, FPF, RF, MMAD and GSD) for samples I and II and different pressure drops.

Sample	I					II				
Cyclone	HPC				SC	HPC				SC
ΔP, kPa	4		2		4	4		2		4
Spray-dried powder:lactose ratio	1:0	1:3	1:0	1:3	1:0	1:0	1:3	1:0	1:3	1:0
EF (%)	92.73 ± 1.24	92.92 ± 0.19	91.05 ± 1.68	92.08 ± 1.85	91.64 ± 3.72	90.96 ± 1.03	93.06 ± 2.40	89.58 ± 3.17	90.55 ± 3.64	94.27 ± 2.69
FPF (%)	27.77 ± 2.51	29.12 ± 1.81	25.15 ± 0.64	26.09 ± 1.01	14.54 ± 0.72	29.09 ± 1.95	32.26 ± 0.58	24.19 ± 1.42	26.78 ± 2.85	19.23 ± 1.79
RF (%)	25.76 ± 2.45	27.05 ± 1.70	22.89 ± 0.22	24.01 ± 1.32	13.34 ± 1.19	26.47 ± 1.93	30.02 ± 0.36	21.69 ± 1.94	25.10 ± 1.95	18.10 ± 1.25
MMAD (μm)	3.19 ± 0.12	3.16 ± 0.49	3.24 ± 0.22	3.22 ± 0.54	3.57 ± 0.16	3.13 ± 0.08	3.11 ± 0.21	3.25 ± 0.07	3.24 ± 0.11	3.35 ± 0.09
GSD	1.87 ± 0.35	2.02 ± 0.19	1.79 ± 0.24	1.87 ± 0.45	2.14 ± 0.44	2.02 ± 0.22	1.92 ± 0.25	1.77 ± 0.08	1.62 ± 0.10	1.83 ± 0.03

Experiments were done in triplicate. ΔP: pressure drop. EF: emitted fraction. FPF: fine particle fraction. RF: respirable fraction. MMAD: mass median aerodynamic diameter. GSD: Geometric Standard Deviation. Alginic-atenolol (Sample I) and alginic-atenolol-potassium (Sample II) microparticles obtained by spray drying using a standard (SC) or a high performance cyclone (HPC).

rier enhances particle deagglomeration and thus improves the emission of particles from the inhaler [57], no statistically significant difference was found between blends with lactose and the carrier-free samples (p-value > 0.05). The addition of the lactose carrier to the SD microparticles led to improvements in FPF and RF between 5–11% and 5–16%, respectively. Although the increase in FPF and RF was incremental, the addition of lactose particles provided better powder flow properties. Considering that DPI formulations aerosolization performances depend on the combination of the drug, the carrier and the inhaler [4], the study of the microparticles interaction with different carriers will be addressed in future works.

All formulations exhibited adequate MMAD for systemic administration (around 3 μm, Table 4). Comparing the blends with lactose and the carrier-free samples, it can be seen that the MMADs are slightly lower for the blends in good agreement with the higher FPF and RF found for the lactose-added samples. The GSD values (1.86–2.15 and 1.77–2.02 for Samples I and II, respectively) demonstrated that in all cases the distributions were narrow (i.e., values well below 3) [31].

The above discussed results indicate that formulations I and II HPC possess adequate aerosolization and deposition properties for different pressure drops and even without using lactose carrier.

3.4.2. Moisture influence on in vitro particle deposition

It has been described that aerosolization and deposition processes could be affected by room humidity conditions to which the DPIs are exposed to, during manufacture, storage and usage, negatively affecting treatment's safety and efficacy [4]. However, the literature has been focused on the effect of the ambient conditions on pure drug. Since in this work the co-processed SD microparticles contain a polymeric material, the study of the influence of the storage RHs on *in vitro* aerosolization and deposition tests is relevant. To this end, Samples I and II HPC with and without the addition of lactose were stored at different relative humidities. After the treatment, the moisture uptake was determined and samples were reassayed in the NGI equipment.

Fig. 2 shows the moisture uptake by SD microparticles with or without lactose as a function of the storage RH. The final water uptake was between 2 and 15%. According to European Pharmacopoeia criteria, the humidity uptake indicated that both powders assayed are moderately hygroscopic [64]. For microparticles blended with lactose, the moisture increase was

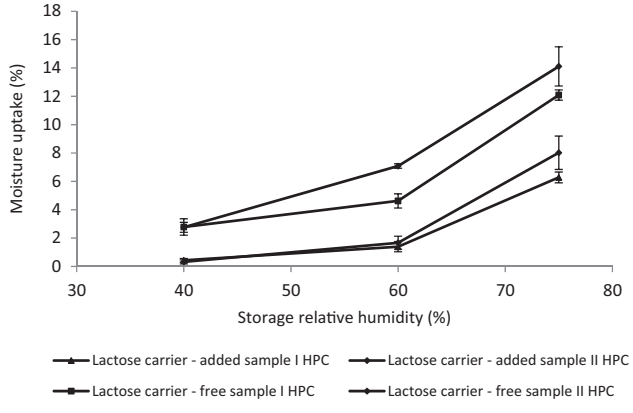

Fig. 2. Percentage of moisture uptake by inhalable microparticles with and without lactose as a function of the storage relative humidity. Alginic-atenolol (Sample I) and alginic-atenolol-potassium (Sample II) microparticles were obtained by spray drying using a high performance cyclone (HPC).

Table 5
NGI deposition parameters for a pressure drop of 4 kPa and samples stored at 40, 60 and 75% of RH.

Sample	I HPC						II HPC					
RH, %	40		60		75		40		60		75	
Spray-dried powder:Lactose ratio	1:0	1:3	1:0	1:3	1:0	1:3	1:0	1:3	1:0	1:3	1:0	1:3
EF (%)	93.16 ± 1.24	94.49 ± 0.72	95.10 ± 1.03	95.31 ± 1.49	84.28 ± 2.30	93.87 ± 1.79	94.28 ± 1.67	94.00 ± 1.65	92.73 ± 1.43	95.04 ± 2.05	80.04 ± 1.18	93.92 ± 3.09
FPF (%)	24.91 ± 1.66	20.83 ± 1.38	23.64 ± 2.81	17.98 ± 2.26	4.05 ± 0.19	3.79 ± 0.33	26.20 ± 1.07	27.37 ± 2.96	22.16 ± 1.62	21.33 ± 1.02	3.76 ± 0.89	3.61 ± 0.79
RF (%)	23.21 ± 1.73	19.69 ± 2.21	22.50 ± 2.90	17.16 ± 2.37	3.59 ± 0.22	3.56 ± 0.29	24.70 ± 1.21	25.75 ± 2.48	20.55 ± 1.50	20.29 ± 1.39	3.01 ± 0.71	3.38 ± 0.73
MMAD (μm)	3.21 ± 0.23	3.28 ± 0.53	3.24 ± 0.36	3.47 ± 0.31	4.37 ± 0.38	4.61 ± 0.47	3.19 ± 0.17	3.15 ± 0.15	3.36 ± 0.28	3.48 ± 0.39	4.86 ± 0.70	5.29 ± 0.21
GSD	2.16 ± 0.35	2.27 ± 0.09	1.87 ± 0.32	2.22 ± 0.17	3.10 ± 0.11	3.13 ± 0.13	2.23 ± 0.11	2.15 ± 0.17	1.78 ± 0.28	2.15 ± 0.13	2.96 ± 0.60	2.55 ± 0.19

Experiments were done in triplicate. RH: relative humidity; EF: emitted fraction; FPF: fine particle fraction; RF: respirable fraction; MMAD: mass median aerodynamic diameter; GSD: Geometric Standard Deviation. Alginic-atenolol (Sample I) and alginic-atenolol-potassium (Sample II) microparticles obtained by spray drying using a high performance cyclone (HPC).

lower than the carrier-free samples due to the low lactose tendency to take ambient moisture [65].

Table 5 shows relevant NGI deposition parameters for Samples I and II HPC, with and without lactose, stored at different RHs and tested at 4 kPa. As it can be seen in Table 5 for RHs of 60 and 75%, FPF and RF were similar for carrier-free or lactose-added materials. For RH of 40%, only the carrier-free Sample II HPC presented FPF and RF similar to the materials containing lactose. Contrarily, Sample I HPC with lactose showed a decrease (about 15%) in the FPF and RF values statistically significant with respect to the sample free of lactose carrier (p-value < 0.05). These results indicate that, at the tested relative humidities, the selected excipient did not improve the material aerosolization.

The results for the lactose carrier-free samples, conditioned at 40 and 60% RH, were also compared to the ones obtained for the same samples stored in a desiccator (Table 4). The statistical analysis indicated that only for Sample II HPC, the observed changes in FPF and RF were statistically significant (p-value < 0.05) when this sample was stored at 60% RH. For Sample I kept at 60% RH and for both samples exposed to 40% RH, the statistical analysis indicated that no significant difference was found for the FPF and RF values corresponding to the conditioned and dried samples (p-value > 0.05). In all cases, the FPF and RF are still adequate for atenolol inhalation administration, moreover if adequate protection from moisture is employed in the product manufacture. However, when samples were stored at 75% RH, FPF and RF sharply decreased by about 90% with respect to dried samples (see Table 5), the exposition to this very high RH should be avoided to protect the product. The decrease in aerosolization properties at 75% RH, could be associated with the swelling capability of the hydrophilic polymer [66].

In the supplementary material, for Sample II as an example, Fig. S1 shows SEM micrographs of the dried and conditioned SD microparticles. Fig. S1a to c, which correspond to Sample II kept in a desiccator and conditioned at 40 and 60%, indicate that the morphology of the samples is quite similar; observation is in good agreement with the results obtained in the NGI assay. However, Fig. S1d (Sample II conditioned at RH of 75%) shows a gelified network, where no individual particles could be recognized.

4. Conclusions

The high performance cyclone allowed collecting smaller particles and improving significantly the SD yield (>80%). The particles produced by using the HPC exhibited better aerosolization performance than the one showed by particles collected by the SC, as consequence of the lower particle mean size. On the other hand, changes in feed dispersions pH did not affect the process yield, product residual moisture or morphology. However, the higher neutralization degree of the polymer decreased the feed viscosity and consequently the size of SD particles. In addition, this change in composition modifies moisture sorption property of the product. The combination of using the HPC and a neutralized feed (Sample II HPC) led to the highest *in vitro* deposition.

The small particle mean size found for the studied formulations explained the poor flow properties, which were improved by diluting the microparticles in lactose. Although this carrier was helpful to handle the particulate systems, its presence did not enhance the powders aerosolization performance. Moreover, the SD powders (with or without lactose) had good *in vitro* deposition (emitted fractions higher than 90% and fine particle fractions higher than 20%, values superior than commercial DPIs), even when they were conditioned up to 60% of RH.

Lactose free-sample II HPC presented fine particle and respirable fractions of 29.1% and 26.5%, respectively. These fractions are in good agreement with the values reported for commercial and in developing formulations, indicating that the proposed alginic acid-atenolol system showed favorable *in vitro* properties for inhalatory administration. Thus, the best formulation (Sample II HPC) is a good candidate to be evaluated in cell cultures and laboratory animals, to prove biocompatibility and bioavailability.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejpb.2016.09.020.

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