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Research Article

Formulation and Characterization of Polysaccharide Microparticles for Pulmonary Delivery of Sodium Cromoglycate

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ABSTRACT. Sodium cromoglycate (SC) is an antiasthmatic and antiallergenic drug commonly used for chronic inhalation therapy; however, many daily intakes are required due to the fast drug clearance from airways. For these reasons, SC polymeric particles for inhalatory administration with adequate aerosolization and mucoadhesive properties were designed to prolong the drug residence time in the site of action. Sodium carboxymethylcellulose (CMCNa), sodium hyaluronate, and sodium alginate were selected to co-process SC by spray drying. The influence of these polysaccharides on the spray drying process and powder quality was evaluated (among others, morphology, size, moisture content, hygroscopicity, flowability, densities, liquid sorption, and stability). *In vitro* aerosolization, drug release, and mucoadhesion performance were also studied. Particularly, a novel method to comparatively evaluate the interaction between formulations and mucin solution (mucoadhesion test) was proposed as a rapid methodology to measure adhesion properties of inhalable particles, being the results as indicative of clearance probability. Among all the studied formulations, the powder based on SC and CMCNa exhibited the best mucoadhesion and aerosolization performance, the highest process yield and adequate moisture content, hygroscopicity, and stability. SC-CMCNa formulation arose as a promising inhalatory system to reduce the daily intakes and to increase the patient compliance.

KEY WORDS: inhaled particles; mucoadhesion; polysaccharides; sodium cromoglycate; spray drying.

INTRODUCTION

The administration of drugs by the inhalatory route constitutes the first-line treatment of local respiratory pathologies such as asthma or chronic obstructive pulmonary disease. Asthma is a chronic respiratory disease characterized by variable and recurring episodes or attacks of impaired breathing (1). The Global Initiative for Asthma estimated that approximately 300 million people suffer from asthma worldwide (2). The inhalatory route allows the direct administration of the drug to the action site, thus decreasing the total dose to be administered (3). Currently, three categories of delivery system are used for aerosolized medications: nebulizers, pressurized metered dose inhalers (pMDIs), and dry powder inhalers (DPIs). The DPIs contain dried microparticles of the drug alone or with carriers (*e.g.*, lactose) and deliver the powders using the inspiratory flow of the patient, thus are propellant-free. Besides, DPIs offer other advantages over nebulizers and pMDIs, such as follows: portable and low-cost device, high formulation stability due to the solid state of the drug, and ease of operation by patients (4). These advantages make DPIs appropriate for both chronic and intensive therapies (5). Even though for the therapeutic success of DPIs, the particles containing the drug require to meet relevant technological challenges: acceptable aerodynamic diameters, adequate dispersion of the particles during the inhalation, and slow clearance in the lung (6). In this sense, the aerodynamic diameters of DPIs' particles should be between 0.5 and 5 μm to achieve a good deposition in the respiratory tract (7). However, particles between these sizes are cohesive and in consequence have poor flowability with a tendency to be retained in the inhaler. Two alternatives are commonly used to improve flowability and dispersion of drug particles: inhaler design improvements and use of drug blends with inert carriers (8). In addition, the inhaled drug may be eliminated from the lung by different mechanisms

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(*i.e.*, mucociliary or cough clearance to the gastrointestinal tract, passive or active absorption into the capillary blood network, metabolism in the mucus or lung tissue, or phagocytosis by alveolar macrophages) and the rates of these phenomena control the residence time of the drug in the respiratory tract (9).

There are several drugs for the treatment of asthma (10). Among them, sodium cromoglycate (SC) is an antiasthmatic and antiallergenic drug commonly used for chronic inhalation therapy (11). SC was studied as a model drug for DPIs formulations because it is a safe drug (12). After inhalation, the SC is well absorbed in the lungs; however, the elimination half-time is about 90–150 min. For this reason, four to eight intakes daily is required (13). For chronic disease treatments, the control of the SC release and clearance rates could increase the therapeutic efficacy, reduce overnight crisis and adverse side effects, and improve the patient compliance. To this end, the incorporation of polysaccharides with mucoadhesive and hydrophilic-swellable properties is a promising strategy to increase the drug residence time (14). Among them, sodium alginate, sodium hyaluronate, and sodium carboxymethylcellulose are good candidates for the therapeutic application described above (15), because they are biodegradable, biocompatible, and safe (16–19).

Either top-down or bottom-up techniques can be used to produce particles with adequate size for pulmonary administration. Among other technologies, milling, spray drying, precipitation, and methods using supercritical fluids can be used (20). Among them, the spray drying technology is a simple, robust, scalable, and relative economic process to generate highly dispersible powders for inhalation in the desirable size range (5,21).

The SC is currently marketed as a fast release DPI containing a 20-mg dose of micronized drug (Intal Spincaps®) (22). Regarding to the production of pure inhalable SC particles, Vidgrén *et al.* (23) observed that spray dried SC powders proved to have better *in vitro* aerosolization properties than the mechanically micronized drug.

For co-processed materials, Vidgren *et al.* (24) found that co-spray dried SC/polyacrylic acid particles showed adequate *in vitro* mucoadhesion; however, this investigation was focused on particles for the nasal route. The particles for this route do not meet the aerosolization and deposition properties that the pulmonary route demands.

Although these are interesting contributions, to our best knowledge, there are no studies related to the development of inhalable and mucoadhesive particles to deliver SC to the lung. For this reason, the novelty of this investigation is the design of SC's co-spray dried particles for inhalatory administration with adequate aerosolization and mucoadhesive properties using some carbohydrate polymers. For this purpose, sodium carboxymethylcellulose, sodium hyaluronate, and sodium alginate were selected. For each formulation, the process yield and several relevant powder properties (*i.e.*, particles' morphology and size, moisture content, hygroscopicity, flowability, densities, structure, thermal behavior, liquid medium sorption, and stability) were evaluated. *In vitro* aerosolization, mucoadhesion by means of a novel method, and drug release rate performance were assessed.

MATERIALS AND METHODS

Materials

The following materials were used as received from the supplier: SC, sodium hyaluronate (HLNa), sieved DC-lactose monohydrate with particle sizes between 77 and 451 μm (Parafarm, Saporiti, Buenos Aires, Argentina), sodium carboxymethylcellulose (CMCNa) medium viscosity with a degree of substitution of 0.7 (Fluka Analytical, Sigma-Aldrich, Argentina), sodium alginate from brown algae (AlgNa) with a mannuronic/guluronic acids ratio of 0.79 (25) (Fluka Analytical, Sigma-Aldrich, Argentina), and mucin from porcine stomach type II (Sigma-Aldrich, Argentina).

Phosphate-buffered saline (PBS, pH 7.4) was prepared by using analytical grade monobasic potassium phosphate and sodium hydroxide (0.2 N).

Methods

Spray Drying (SD): Feed Solution Properties and Operating Conditions

The components used to prepare the aqueous solutions to be spray dried were SC and the selected polysaccharides (CMCNa, AlgNa, and HLNa). The SD feeds were prepared by dissolving 1 g of SC and 0.16 g of polysaccharide with distilled water to a final volume of 100 mL. The polysaccharide content was selected to obtain SD feed solutions with relative low viscosity, favoring the production of microparticles of appropriate sizes for inhalatory route.

The viscosities of the feed solutions were determined using a capillary Cannon-Fenske Routine-type viscometer (Tube size 100, IVA Cannon Instrument Company, State College, USA) immersed in a bath at constant temperature of 25°C. Also, for comparative purposes, the viscosity of polysaccharides solutions without SC was evaluated. Measurements were taken in triplicate.

Solutions containing drug and polysaccharides were fed to a Mini Spray Dryer B-290 (BÜCHI, Flawil, Switzerland). A two-fluid nozzle with a cap-orifice diameter of 0.5 mm was used. The atomizing air pressure was kept constant at 6 bar for all the experiments. After preliminary experiments, the following operating conditions values were selected: inlet air temperature of 110°C, atomization air volumetric flow rate about 600 L/h, feed volumetric flow rate of 6 mL/min, and drying air volumetric flow rate of 35 m^3/h. One hundred milliliters samples containing SC/polysaccharides or pure SC was spray dried in triplicate.

The produced spray dried powders were transferred to tight closed amber glass containers and stored at 45% relative humidity (RH) and room temperature (25°C) (26).

Process Yield (PY)

The process yield was calculated as the ratio of the weight of spray dried powder collected after every SD experiment to the initial solid content in the feed solution.

Moisture Content (MC)

Powders' moisture content was determined by using a moisture analyzer with halogen heating (model M45, Ohaus, Pine Brook, USA). The moisture content was measured immediately after the SD step. About 500 mg of powder was heated up to 70°C until the weight change was less than 1 mg in 90 s. This temperature is well below the degradation temperatures of the SC (27) and the polysaccharides (28–30).

Hygroscopicity (HYG)

Hygroscopicity was determined at 25°C and a relative humidity of 45%. Spray dried samples (approximately 0.1 g) were stored in a closed container and a glycerol-water mixture (83%, *v/v*) was used to provide the selected RH (31). The samples were weighted every day until constant weight was reached. The equilibrium hygroscopicity was expressed as the weight increase per 100 g of dry solids.

Bulk (D_b) and Tapped (D_t) Densities

In order to determine the density of spray dried samples, the powder was gently poured into a 10-mL graduate cylinder. Bulk density (D_b) was calculated as the ratio between the sample weight and the volume occupied. Tapped density (D_t) was estimated as the ratio between the sample weight and the final volume after tapping the cylinder until volume changes were not noticed. Powder compressibility was evaluated using the Carr's compressibility index (CI) (32):

$$\mathrm{CI} = \frac{(D_t - D_b)}{D_t} 100 \tag{1}$$

Particles' Morphology and Size Distribution

The spray dried samples were dried under air flow on a porthole. Afterwards, the samples were metalized with gold in a sputter coater (PELCO 91000, TellPella, Canada). Particles' morphology was assessed using a scanning electron microscope (SEM, EVO 40-XVP, LEO, Oberchoken, Germany).

Particle size distribution (PSD) was measured using a laser light diffraction instrument by means of the dry powder method (LA 950 V2, Horiba, Kyoto, Japan). The spray dried powders were dispersed in lactose monohydrate (with a known particle size distribution between 77 and 451 μm) in a proportion lactose/sample 10:1 to improve the sample flow from the feed hopper to the measuring cell. Not overlapping size distributions were obtained, being then possible to establish the SD-powder particle size distribution precisely (21,33). Average particle size was expressed as $D_{4,3}$ (*i.e.*, mean volume diameter) and the distribution width was reported as span. The span index was calculated as follows:

$$\mathrm{Span} = (D_{90} - D_{10})/D_{50} \tag{2}$$

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 PSD can be considered relatively narrow if the span index is lower than 2 (34).

With the purpose to investigate the effect of the selected storage conditions (45% RH and room temperature) on particles' size, the PSD was again measured after subjecting the SD powders to these conditions for 12 months.

In Vitro Spray Dried Powders' Aerosolization Properties

The *in vitro* aerosolization performance of the SD powders was evaluated in a Next Generation Impactor (NGI, Copley Scientific) equipped with an induction port (IP) and a pre-separator (PS). The cascade impactor employed is a high performance particle classifying cascade impactor comprising seven-stage inertial impactor that separates the powder into ranges of aerodynamic diameters and, as a final stage, by a micro-orifice collector (MOC) (35). The instrument provides useful information regarding particle size (*i.e.*, allows the measurement of the aerodynamic particle size distribution) and offers a guide to particle deposition in the respiratory tract (36,37).

Size 2 gelatin capsule loaded with the powder was placed into a Breezhaler® dry powder device (Novartis) connected *via* a mouthpiece adapter (MA) to the IP. For the assays, about 16 mg of SD powders and 50 mg of lactose monohydrate were blended.

The blends constituted by the SD powders and the carrier were aerosolized at 60 L/min for 4 s and the assay was done in triplicate. For selected flow rate, the aerodynamic cutoff diameters for each stage of the impactor are as follows: stage 1 (8.06 μm); stage 2 (4.46 μm); stage 3 (2.82 μm); stage 4 (1.66 μm); stage 5 (0.94 μm); stage 6 (0.55 μm); and stage 7 (0.34 μm) (35).

To prevent particle re-entrainment, the NGI stages were precoated with glycerin (38,39). After aerosolization, the powders deposited on the capsule, device, MA, IP, PS, NGI-1 to 7 stages, and MOC were recovered with an appropriate volume of water. The drug content within each sample was determined by UV spectrophotometry at 326 nm (UV-160A, Spectrophotometer, Shimadzu, Burladingen, Germany). The analytical method was based on the one given for SC by the USP 30-NF 25 (32). In addition, solutions of the selected polysaccharides were prepared to test their UV absorption ability. These measurements showed that the presence of polysaccharides did not affect the SC quantification by UV.

According to Donovan and Smyth (40), the Eqs. 3, 4, and 5 were used to calculate the emitted fraction (EF), fine particle fraction (FPF), and respirable fraction, respectively.

$$\mathrm{EF}\% = \frac{\text{drug mass deposited on IP, PS and all the NGI stages}}{\text{total drug recovered}} \times 100 \tag{3}$$

$$\mathrm{FPF}\% = \frac{\text{drug mass deposited on stages 3–7 and MOC}}{\text{drug mass deposited on IP, PS and all the NGI stages}} \times 100 \tag{4}$$

$$\mathrm{RF}\% = \frac{\text{drug mass deposited on stages 3–7 and MOC}}{\text{total drug recovered}} \times 100 \tag{5}$$

The mass median aerodynamic diameter (MMAD) was calculated from a drug mass cumulative distribution (built

considering the drug mass collected in NGI-1 to 7 stages and MOC) and was defined as the diameter at which 50% of the drug is collected in larger particles and the remaining 50% is collected within smaller particles. 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 was recovered from the NGI-1 to 7 stages and MOC, respectively.

In Vitro Mucoadhesion: Tensile Strength

Currently, there is no available standardized *in vitro* method to investigate mucoadhesion of polymers and pharmaceutical dosage forms. Numerous methods have been proposed, and the most used apparatus is the Texture Analyzer (41). In the present work, a TA Plus texture analyzer (Lloyd Instruments, England) equipped with a 5-kg load cell was used to study the influence of the selected polysaccharides on the mucoadhesion of SD particles. The apparatus consists of a stationary surface in which a filter paper (2.5 mm diameter with impermeable back) was attached by using a double-sided adhesive tape. A mucin solution (3 wt%) of 0.1 mL in PBS maintained at 37 ± 0.5°C was placed over the filter paper and allowed to stand for about 15 min. The mucin concentration was selected because in conducting zone of lung (*i.e.*, secondary bronchi, bronchioles and terminal bronchiole) the mucin concentration is usually within 2–4% (17). The choice of an appropriate mucosal medium for mucoadhesion measurement is still under discussion. The use of animal mucosal tissue has demonstrated to provide mucoadhesion results with high standard deviations (41). Therefore, mucin isolated from porcine stomach was commonly used to establish mucoadhesion properties. Hassan and Gallo (42), Rossi *et al.* (43), Tamburic and Craig (44), Hagesart and Sande (45), and Ivarsson and Wahlgren (46) studied the *in vitro* mucoadhesive behavior using different polymeric compounds and porcine gastric mucin. They concluded that this mucin was satisfactory for mucoadhesion tests. Therefore, mucin from porcine stomach was selected for the mucoadhesion test of the developed formulations.

Above the stationary surface, there was a movable probe on which the SD particles were also attached by a double-sided adhesive tape. The particle's attachment was performed by immersing the probe into a powder bed. Thereafter, the probe was gently shaken to remove any excess of powder to achieve a monolayer of particles (47). Then, the movable probe was lowered until the particles soaked with the mucin solution for 3 min (no force was applied) and at that time the probe was raised at a rate (*i.e.*, withdrawal speed) of 0.1 mm/s and the maximum detachment force (MDF) was measured using the computer software (Nexygen Plus) (46). Eight MFD measurements were made on each sample (*i.e.*, SD powders and pure polymers). In addition, the mucin solution was evaluated using the probe free of powder.

Prior to selecting the operating parameters for the mucoadhesion test (mentioned above), preliminary experiments were performed to obtain MDFs higher than 0.5 N. This minimum value is recommended to obtain reliable results (48). To this end, the SC:CMCNa powder was used and different levels of the following parameters were evaluated: (a) drying time of mucin solution (before starting the test): 0, 5, and 15 min; (b) volume of mucin solution: 0.1, 0.2, and 0.4 mL; (c) withdrawal speed of the upper probe: 0.01, 0.05, 0.1, and 0.15 mm/s; and (d) initial contact time between the particles and the mucin solution: 3, 6, 9, and 12 min. The parameters were modified one at a time, adequate MDFs values were found for the optimized conditions above described.

Release of SC from SD Powders

Currently, no official methodology exists for the evaluation of the *in vitro* release rates from inhalable drugs. Different methodologies were used such as follows: flow through apparatus, diffusion apparatus (*i.e.*, modified Franz diffusion cell), and USP dissolution apparatus type II. For the last methodology, the particles can be either added directly into the dissolution medium, or into dialysis bags attached to the apparatus paddles, or in a membrane holder directly taken from the impactor stages (49,50). In the present work, drug release experiments were carried out using a method based on the USP dissolution test using a dissolution apparatus II (708-DS, Dissolution Apparatus, Agilent Technologies, Santa Clara, USA). The selected methodology and the dissolution parameters were based on the dissolution study of spray dried SC microparticles carried out by Salama *et al.* (51). The volume of dissolution medium (PBS) was 500 mL at 37°C with stirring at 50 rpm. For each SD powder, samples containing about 80 mg of SC to simulate a daily dose were employed (52). At predetermined time intervals, samples of 5 mL were withdrawn and SC was determined spectrophotometrically at 328.5 nm (maximum absorption wavelength of the drug in PBS). The same volume of fresh medium at 37°C was subsequently added into the vessel after each withdrawal (Agilent 8000, Dissolution Sampling Station, Agilent Technologies, Santa Clara, USA). Finally, the release profiles from the different SD powders were compared using the similarity factor, f_2, calculated by Eq. (6):

$$f_2 = 50\log\left\{\left[1+\left(1\middle|q\right)\sum_{t=1}^{q}(R_t-T_t)^2\right]^{-0.5}100\right\} \tag{6}$$

where q is the number of dissolution time points, and R_t and T_t are the percentages of drug released at each time. An f_2 value between 50 and 100 indicates similarity between the two release profiles (53).

Powders' PBS Uptake

The SD powder wettability was evaluated by measuring the phosphate-buffered saline uptake using a similar device to the one developed by Nogami *et al.* (54). The apparatus was a U-shaped tube which had at one extreme a holder with a porous glass base and the other end was connected to a graduated pipette horizontally oriented and calibrated at the same level of the holder base. A precise weighed amount of the SD powder (containing 80 mg of SC) was placed in the holder (time zero) and the material began capturing PBS by capillarity. The volume of PBS sorbed by the sample was

measured following the water displacement in the pipette as a function of time. All the assays were performed in triplicate at room temperature. In order to evaluate the SD powders' sorption, the data was analyzed considering the uptake model given by Vergnaud (55):

$$M_t = kt^n \tag{7}$$

where M_t is the mass uptake at time t, k the swelling constant, and n is related to the mechanism of PBS uptake. A value of $n \leq 0.5$ indicates a diffusion-controlled mechanism in which the rate of diffusion of the liquid is lower than the rate of relaxation of the polymer chains. On contrary, $n = 1$ suggests that the relaxation process is very slow as compared with the rate of liquid diffusion. This means that the liquid diffuses through the polymer matrix at a constant velocity. A value of n between 0.5 and 1 indicates that the rate of diffusion of the liquid and that of relaxation are of the same magnitude (56).

Thermogravimetric Analysis (TGA)

The weight loss of each formulation as a function of temperature was determined using a thermogravimetric analyzer (Discovery, TA Instruments, New Castle, USA). About 5 mg of each powder was weighed and heated at 10°C/min under nitrogen purge from 50 to 350°C (27).

Glass Transition Temperature (T_g) Determination

The glass transition temperature (T_g) of the powders was measured by differential scanning calorimetry (Pyris 1, Perkin Elmer, Massachusetts, USA). Selected samples of 10 mg were placed in aluminum pans and scanned from 25 to 200°C at a heating rate of 10°C/min. The purpose of this first thermal scan was to remove the residual moisture that could affect the determination of T_g. Then, the samples were cooled down from 200 to 25°C at 10°C/min and re-heated from 25 to 200°C at the same rate. T_g was calculated from the thermograms as the temperature at which one half of the change in heat capacity, ΔCp, occurred (*i.e.*, by the half ΔCp method) (57).

X-Ray Diffraction (XRD)

XRD patterns of the spray dried products were recorded using a Rigaku Geigerflek (DMAX 3C, Tokyo, Japan). X-ray diffraction system and the software JADE 7 were used to identify crystalline materials. The anode X-ray tube was operated at 35 kV and 15 mA. Measurements were taken from 2° to 60° on the 2θ scale at a step size of 4°/min. The measurements were performed right after the spray drying step (powders' samples as produced, *i.e.*, without any further conditioning). In addition, in order to explore the effect of the selected storage conditions (45% RH and room temperature) on the powder structure, the assay was again performed after the powders were stored for 12 months.

Statistical Analysis

Significant differences between formulations were performed using one-way ANOVA followed by LSD multiple comparison. A p value <0.05 was considered to be significant.

RESULTS

Spray Drying Performance

For all the spray dried powders, the obtained process yields were higher than 55% (Table I), values more than acceptable for the laboratory scale dryer (58). In addition, the outlet temperatures for all the samples were in the range of 74–75°C, these values were well below from the drug and polysaccharides thermal temperature degradation (see "Stability of the SD particles"). Besides, the spectrophotometric measurement of the drug content in the co-processed powders showed that the amount of SC in the produced particles was in concordance with the expected theoretical value (see supplementary material Table S1), result that indicated that no selective stickiness in the spray dryer chamber walls occurred (33).

Even though all the samples exhibited good process yield values, the statistical analysis showed significant difference between them ($p < 0.05$). In this sense, an inverse relationship between process yield and feed viscosity values was found (see Table I). The feed solution containing pure SC exhibited the highest product recovery and the lowest viscosity. The polysaccharide's addition increased the feed viscosity (in good agreement with the viscosities exhibited by the polysaccharides solutions without SC, see supplementary material Table S2) and decreased the process yield. The same behavior was observed by Ceschan *et al.* (33) when co-processed by SD atenolol with alginic acid. The authors observed that higher alginic acid contents increase the feed viscosity and consequently diminished the process yield. Other authors, such as Rabbani and Seville (59), produced powders for inhalation administration by spray drying solutions with different ethanol contents, β-estradiol, lactose, and leucine. These researchers also observed that the process yield decreased as the feed viscosity increased.

SD Particles Characterization

Moisture Content and Hygroscopicity

For all the SD powders, the moisture content was lower than 7.20% (Table I), common values for spray dried SC powders (60). The statistical analysis showed significant difference between the powders' moisture content ($p < 0.05$). The co-processed materials containing polysaccharides exhibited lower moisture content than SC spray dried powder. As it was expected, the higher the solid content in the feed, the lower final moisture content (see Table I) (61,62).

The spray dried powders of pure SC have been reported to be hygroscopic materials (63). For this reason, the samples were conditioned at 45% RH and room temperature (64). The SD powder containing pure SC showed a hygroscopicity value of 8.73 ± 0.08% (Table I); this equilibrium value was lower than the one reported by Salama *et al.* (26) for pure SC spray dried stored

Table I. Process Yield (PY), Feed Viscosity, Powder Moisture Content (MC), Hygroscopicity (HYG), Characteristics Particle Diameters (D_{50}, D_{10}, and D_{90}) and PSD Span of SD Samples Containing Pure SC and Co-processed Formulations (SC:CMCNa, SC:HLNa, and SC:AlgNa). All the Results Are Expressed as Mean ± SD

Sample	PY (%)	Feed viscosity ($mm^2\ s^{-1}$)	MC (%)	HYG (%)	D_{50} (μm)	D_{10} (μm)	D_{90} (μm)	Particle size distribution span
SC:CMCNa (1:0.16)	60.66 ± 2.83	3.74 ± 0.03	6.15 ± 0.05	8.01 ± 0.12	5.28 ± 0.03	2.95 ± 0.03	8.88 ± 0.14	1.12 ± 0.02
SC:HLNa (1:0.16)	55.50 ± 1.15	9.20 ± 0.01	6.78 ± 0.20	7.59 ± 0.03	5.27 ± 0.30	3.09 ± 0.12	8.54 ± 0.68	1.03 ± 0.05
SC:AlgNa (1:0.16)	60.38 ± 1.06	4.68 ± 0.03	7.01 ± 0.12	9.81 ± 0.04	5.08 ± 0.04	2.96 ± 0.22	8.69 ± 0.50	1.13 ± 0.05
SC (1:0)	61.22 ± 0.77	1.44 ± 0.01	7.20 ± 0.09	8.73 ± 0.08	5.34 ± 0.27	2.86 ± 0.11	9.94 ± 0.65	1.21 ± 0.04

PY process yield, *MC* moisture content, *HYG* hygroscopicity, *SC* sodium cromoglycate, *CMCNa* sodium carboxymethylcellulose, *HLNa* sodium hyaluronate, *AlgNa* sodium alginate

at the same conditions. Regarding the hygroscopicity of the co-processed materials (Table I), the values are significantly different ($p < 0.05$). The differences, even when all the feed formulations were dried using the same SD operating conditions, can be attributed to the presence of different polysaccharides used in the tested formulations. The incorporation of HLNa and CMCNa produced co-processed powders with lower hygroscopicity values than the spray dried pure SC powder. Also, these powders showed the lowest moisture content values (Table I). When AlgNa is co-processed with SC, the powders exhibited the highest hygroscopicity and moisture content values (Table I). The moisture content after conditioning allowed the proper manipulation of all the powders, then from this practical point of view, the hygroscopicities of the SD powders can be consider low.

Particle Morphology, Size, and Densities

The SEM micrograph (Fig. 1a) showed that the particles obtained by SD of the pure drug exhibited a mushroom shape and a roughness surface, a typical shape found for SC spray dried (Nolan *et al.* (22)).

As all the co-processed powders were obtained at the same operating conditions and solid concentrations, the particle's morphology differences between the formulations should be attributed to the use of different polysaccharides. The SEM micrographs (see Fig. 1b–d) indicated that as the viscosity of the SD feed becomes higher (see Table I), the particles are more spherical and with a more homogeneous surface morphology. Regarding the relationship between particle morphology and feed viscosity, Barron *et al.* (65) reported that high viscosities exert a stabilizing effect on atomization process by opposing the onset turbulence when the solution was atomized; thus, a major tendency to obtain spherical particles is expected.

Concerning particle size distribution, the statistical analysis showed no significant difference ($p > 0.05$) between the mass medians (D_{50}). These values were between 5.08 ± 0.04 and 5.34 ± 0.37 μm (Table I). D_{10}, D_{90}, and span values are also shown in Table I. For all the powders, the span values were close to 1, suggesting that, for the selected SD operating conditions, narrow size distributions were produced regardless the polysaccharide type.

The bulk and tapped density values were between 0.279–0.327 and 0.386–0.470 g/cm^3, respectively (Table II). The statistical analysis showed no significant difference ($p > 0.05$) between the powders' densities.

Carr's compressibility indexes of 10% indicate an excellent flow, between 11 and 15% denote good flowability, between 16 and 20% reveal fair flow, between 21 and 25% indicate acceptable flow, and between 26 and 31% are an indication of poor flow (32). For all the spray dried powders, the CI values were in the range of 28.52 ± 1.28 and 33.33 ± 1.80%, indicating poor flowability (Table II). The statistical analysis showed significant difference between the CI values, the SC:AlgNa particles showed the worst flow. The observed cohesiveness can be attributed to interparticle forces, which are affected by various particles' properties, such as size, morphology, and density (27).

Powder X-Ray Diffraction

The XRD measurement of the raw SC revealed a crystalline structure (Fig. 2a). In contrast, the spray dried SC powder showed a completely amorphous state, as it can be confirmed by the presence of broad non-defined peaks with abundant noises (Fig. 2a). These results were in well agreement with the X-ray diffraction patterns observed by Najafabadi *et al.* (27) for both commercial and spray dried SC.

The pure CMCNa and AlgNa polysaccharides exhibited amorphous states (Fig. 2b). On the other hand, HLNa showed an amorphous halo together with a defined peak indicative of some degree of crystallinity (Fig. 2b). For this material, depending on the isolation technique, different degrees of crystallinity can be found (67).

Regarding the co-processed powders, independently of the polysaccharide type, all the formulations exhibited a completely amorphous state (Fig. 2c).

In Vitro Pharmaceutical Performance of the SD Powders

Spray Dried Powders' In Vitro Aerodynamic Properties

Relevant drug fractions for inhalable particles, the MMAD and GSD are presented in Table III. The emitted fractions of all the co-processed powders were higher than 86.96%, while the powder containing CMCNa showed the highest values of FPF and RF. Nolan *et al.* (22) studied the aerosolization parameters of micronized pure SC commercially available (Intal ®) using an Andersen cascade impactor. In that study, the EF value was 63%, well below the EF value found for co-processed powders obtained in the present work (Table III).

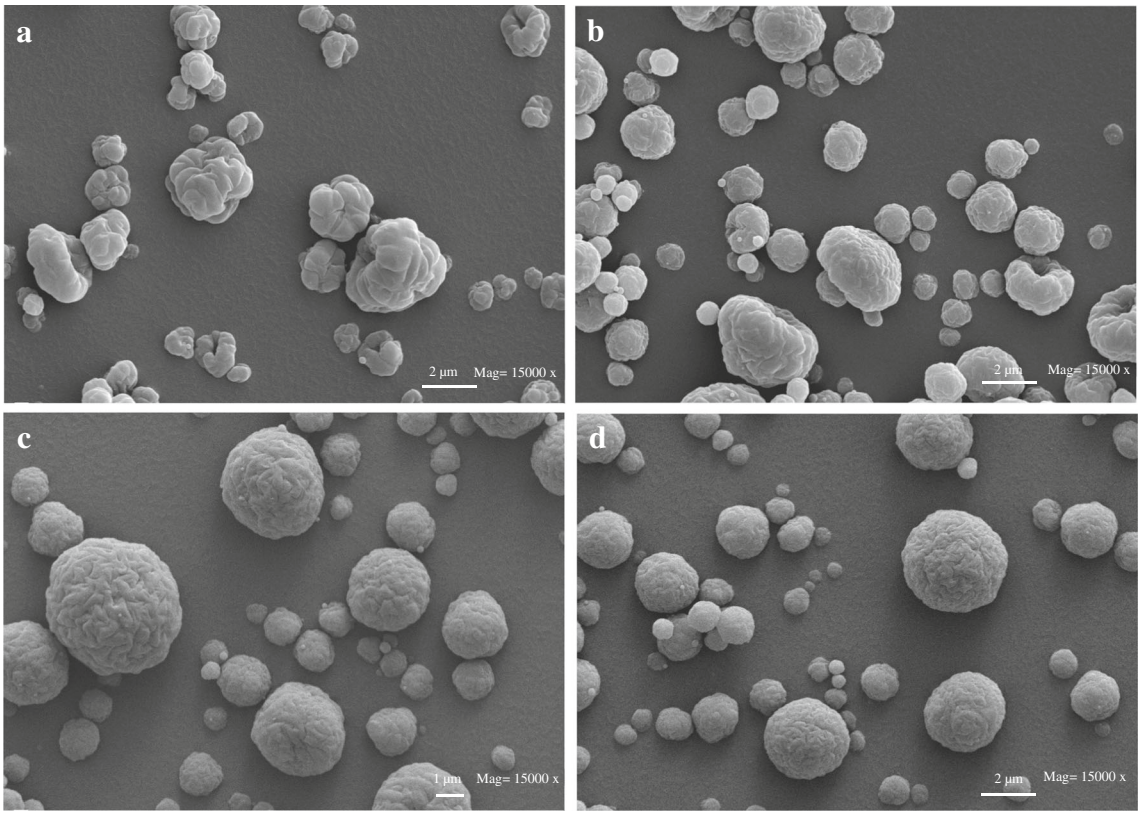

Fig. 1. SEM micrographs (15,000×) of **a** SC spray dried and co-processed powders: **b** SC:CMCNa; **c** SC:AlgNa; and **d** SC:HLNa

Regarding the FPF, defined as the drug content in particles lower than 5 μm respects to the inhaler output, the powder based on CMCNa showed the highest FPF value (Table III). Since Eq. (4) cannot be directly compared with the FPF reported by Nolan *et al.* (22) for the commercial SC DPI, for comparison purposes a new fraction accounting for the drug mass in particles lower than 4.46 μm respects to the capsule output was calculated. This value (in spite of the proposed fraction calculation provides a conservative value with respect to the one informed by Nolan *et al.* (22)) was about 35% for the SC:CMCNa powder and well above the FPF reported for the commercial product (28%).

The MMADs for all the formulations were similar and lower than 5 μm, suggesting their ability to adequately deposit throughout all regions of the lungs and effectively reach the lower airways. Regarding the geometric standard deviation, all the formulations presented GSDs between 1.66 and 1.86. Since the values were lower than 3, in agreement with the calculated PSD span values (see Table I), all the aerodynamic size distributions can be considered as narrow ones (19). For these parameters, Nolan *et al.* (22) found higher MMAD (7.6 μm) and GSD (2.7) than the co-processed powders obtained in the present study. Summarizing, the SC:CMCNa product displayed the best aerosolization properties. Therefore, this material showed good attributes for inhalatory administration even better than the micronized pure SC commercially available.

In addition, Vidgren *et al.* (66) compared the deposition of spray dried pure SC based on *in vitro* (by using a cascade impactor) and *in vivo* (by gamma camera in healthy human volunteers) tests. The authors found a 39% of drug (7.8 mg) deposited in the impactor stages between 0.3 and 7.1 μm. In correlation, a 16% of SC was found to be deposited in the whole lung area in the volunteers (around 3.2 mg), being reported as a proper value for therapeutic action. In our work, 53% of the drug (about 8.5 mg) was obtained for SC:CMCNa powder in the stages above mentioned. Therefore, this result suggests that the formulation would have an adequate *in vivo* deposition.

Table II. Spray Dried SC and Co-processed (SC:CMCNa, SC:HLNa, and SC:AlgNa) Powders' Bulk and Tapped Densities and Carr's Compressibility Index. All the Results Are Expressed as Mean ± SD

Sample	D_b (g/cm^3)	D_t (g/cm^3)	CI (%)
SC:CMCNa (1:0.16)	0.307 ± 0.011	0.443 ± 0.022	30.64 ± 1.06
SC:HLNa (1:0.16)	0.327 ± 0.012	0.470 ± 0.012	30.42 ± 0.72
SC:AlgNa (1:0.16)	0.313 ± 0.004	0.470 ± 0.013	33.33 ± 1.80
SC (1:0)	0.279 ± 0.016	0.386 ± 0.016	28.52 ± 1.28

CI Carr's compressibility index, *SC* sodium cromoglycate, *CMCNa* sodium carboxymethylcellulose, *HLNa* sodium hyaluronate, *AlgNa* sodium alginate

In Vitro Mucoadhesion Test

The MDFs by using the mucin solution and the spray dried powders and the pure polysaccharides are shown in Fig. 3. The reference measurements (mucin solution), as

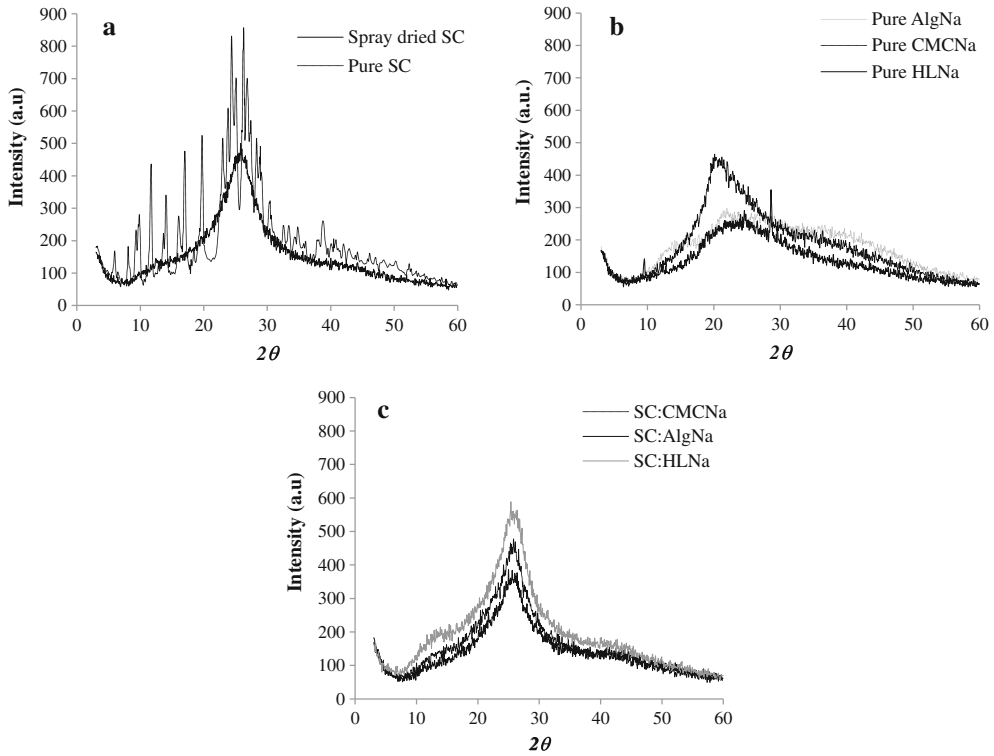

Fig. 2. Diffractograms: **a** pure and spray dried drug; **b** pure polysaccharides; and **c** spray dried co-processed particles

expected, showed the lowest MDF. The co-processed powders presented higher MDFs than the pure spray dried SC; thus, the mucoadhesion improvement can be attributed to the presence of polysaccharides as it can be inferred from the MDF values for pure polysaccharides. The SC:CMCNa powder showed the highest MDF value of the co-processed materials.

The statistical analysis showed significant difference between the MDFs for all the materials ($p < 0.05$). The LSD multiple comparison procedure was applied to determine which means were significantly different from others. For the co-processed powders, the pair composed by SC:AlgNa/SC:HLNa showed no statistically significant difference. On the other hand, the pairs SC:CMCNa/SC:AlgNa and SC:CMCNa/SC:HLNa showed statistically significant differences. Therefore, the incorporation of CMCNa in the powder produced a relevant effect in the material mucoadhesion performance.

In Vitro Dissolution and PBS Uptake

As mentioned above, the particle median size measured by laser diffraction exhibited similar values for all the formulations. For this reason, the external surface areas of all the co-processed powders are expected to be similar; therefore, differences in the drug dissolution rate should be attributed to the different chemical composition of the formulations. Figure 4 shows the dissolution profiles of the co-processed powders and the SD pure SC (the profile for the first 15 min was amplified to observe the initial drug release in more detail). The spray dried SC powder exhibited a complete drug release within the first 6 min. On the other hand, the co-processed powders showed slower drug release rates than the spray dried SC. SC:HLNa and SC:AlgNa powders showed a complete drug release after 15 min (Fig. 4). Instead, SC:CMCNa particles exhibited the highest time to reach the total release of the drug (*i.e.*, 60 min) (Fig. 4). The

Table III. The Emitted Fraction (EF, %), Fine Particle Fraction (FPF, %), Respirable Fraction (RF, %), Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation (GSD) of Spray Dried Co-processed Particles Obtained from Aerosolization Analysis by NGI. All the Results Are Expressed as Mean ± SD

Sample	EF (%)	FPF (%)	RF (%)	MMAD (μm)	GSD
SC:CMCNa (1:0.16)	90.18 ± 4.97	37.62 ± 2.75	33.84 ± 0.94	4.09 ± 0.23	1.76 ± 0.07
SC:HLNa (1:0.16)	90.40 ± 2.84	28.53 ± 4.17	25.72 ± 3.06	4.21 ± 0.15	1.86 ± 0.06
SC:AlgNa (1:0.16)	86.96 ± 1.92	31.05 ± 1.97	26.97 ± 1.14	4.16 ± 0.19	1.66 ± 0.03

EF emitted fraction, *FPF* fine particle fraction, *RF* respirable fraction, *MMAD* mass median aerodynamic diameter, *GSD* geometric standard deviation, *SC* sodium cromoglycate, *CMCNa* sodium carboxymethylcellulose, *HLNa* sodium hyaluronate, *AlgNa* sodium alginate

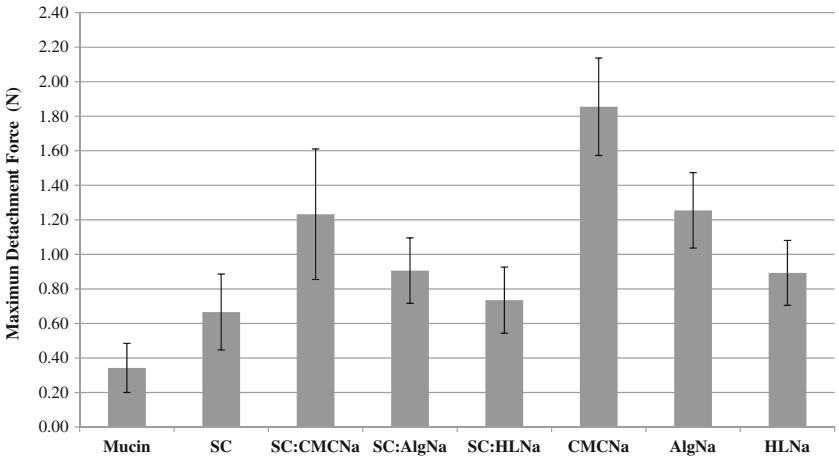

Fig. 3. Tensile strength measurements for mucoadhesion evaluation: maximum detachment force (MDF) measured by Texture Analyzer of mucin solution, spray dried drug, co-processed particles, and pure polysaccharides

similarity factor (f_2) was used to compare the powders' dissolution profiles. As expected, this parameter revealed no similarity between the spray dried SC and the co-processed powders. For the materials containing HLNa and AlgNa, f_2 values were higher than 50, indicating their similar dissolution profiles. On the other hand, the comparison between the CMCNa-based powder and the other co-processed products showed f_2 values lower than 50, indicating no similarity between these dissolution profiles. Clearly, by using the same polysaccharide content, the CMCNa delayed the SC release rate in comparison to HLNa and AlgNa.

In order to understand the drug dissolution behavior from the different co-processed powders, the PBS uptake test was performed to characterize powder wetting (Fig. 5). SC:HLNa and SC:AlgNa powders allowed a faster initial rate of liquid incorporation than SC:CMCNa particles (Fig. 5). In concordance, the fitting of the model of Vergnaud (55) indicated that the lowest n values corresponded to the SC:HLNa and SC:AlgNa powders (0.410 and 0.408, respectively). These results indicated that the liquid diffusion rate was slower than the rate of polymer chains relaxation, *i.e.*, the erosion of the polymers gel layer took place. This result is in

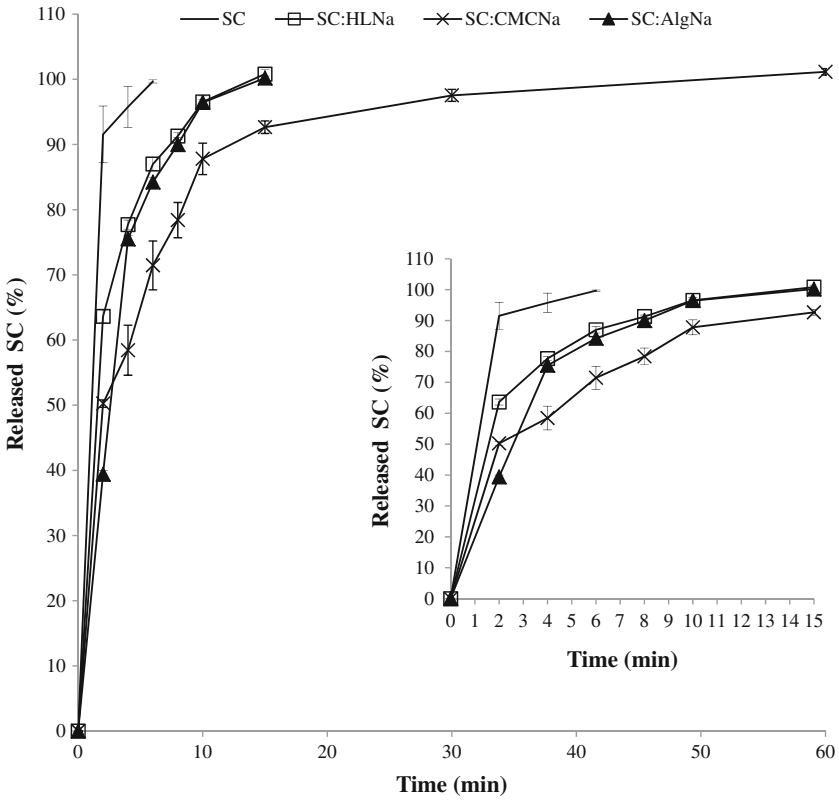

Fig. 4. *In vitro* drug release profiles from the spray dried drug particles and co-processed powders (mean ± SD, $n = 3$)

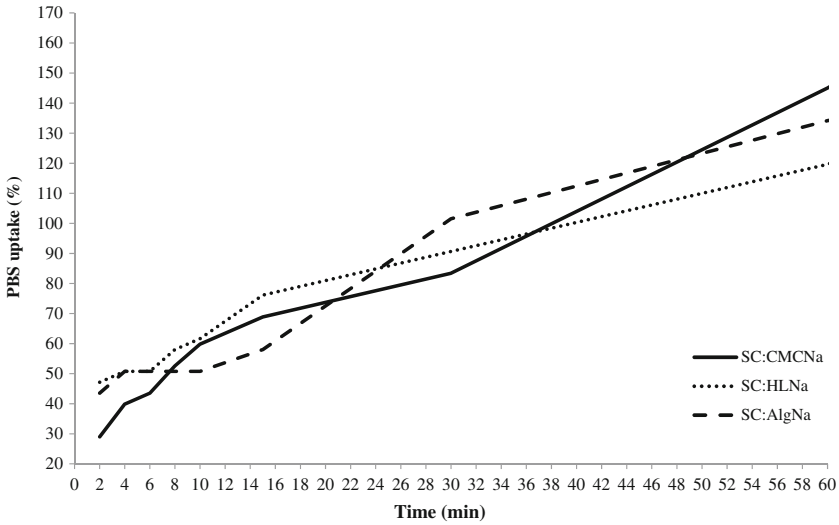

Fig. 5. Phosphate-buffered saline uptake percentage by spray dried co-processed particles (mean ± SD, $n = 3$)

good agreement with the fact that these powders exerted a limited control of the drug release. On the other hand, for SC:CMCNa powder, the *n* value was 0.609, indicating that the liquid diffusion and the polymer chains relaxation rates were similar. Therefore, it expected expansion and disentanglement of the CMCNa chains, forming a viscous gel layer on the particle's surface (visually observed during the PBS uptake assay) which retarded the drug release (68).

Stability of the SD Particles

The T_g values for SC, SC:CMCNa, SC:HLNa, and SC:AlgNa spray dried powders were 118.6, 115.4, 130.9, and 129.7°C, respectively. Since these values are well above room temperature, the powders can be considered stables. In addition, the TGA measurements indicated that thermal decomposition process for all products occurred at temperatures higher than 230°C (see supplementary Fig. S1). These values were in concordance with the ones reported by Najafabadi *et al.* (27) for spray dried SC powder from an aqueous solution.

The powders were stored at 45% RH and room temperature in hermetical containers for 1 year. After this conditioning period, the powder's structure and the particle size were reevaluated. The X-ray patterns showed amorphous structure (data not shown) same with the initial powder's structure (see Fig. 2); thus, crystallization did not take place at the evaluated storage conditions. The characteristics diameters (D_{10}, D_{50}, and D_{90}) were also measured (see supplementary Table S3). The particle size distributions after storage did not suffer significant size changes, the span of all the conditioned powders lower than 1.5.

CONCLUSIONS

This work demonstrated that the co-processed SC:CMCNa powder showed good aerosolization properties in comparison with the commercial formulation. Among the produced co-processed powders, the SC:CMCNa particles presented the highest *in vitro* mucoadhesion. Besides, CMCNa was the polysaccharide that most delayed the SC release.

Regarding the product production and storage, the SC:CMCNa formulation had high process yield, adequate moisture content and hygroscopicity values, and appropriate stability properties.

Therefore, the SC:CMCNa powder arises as an attractive mucoadhesive inhalable SC formulation. Even though this material is a promising product, further *in vivo* studies are needed to demonstrate prolonged residence time of SC in the lung.

Also, a novel method to test *in vitro* mucoadhesion for the initial stages of inhalable particles development was proposed.

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