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Research paper

Physical characteristics and aerosol performance of naringin dry powders for pulmonary delivery prepared by spray-drying

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ABSTRACT

The aim of the present work was to develop dry powders containing naringin for a direct administration to the lung to combat oxidative stress. Naringin microparticles were prepared by spray-drying the neat flavonoid (2–5% w/v) from different water/ethanol co-solvents. The spray-dried powders were characterised for morphology, density, particle size distribution, residual humidity, crystallinity, solubility, thermal behaviour and respirable fraction.

The fine fraction of the powders was measured by single-stage glass impinger and Andersen cascade impactor, using the Turbospin® device for the deposition tests, wherein the dose to be aerosolised was premetered in a gelatine capsule.

By increasing the ethanol content, the feed liquid turned from a suspension into a solution: the spray of flavonoid suspensions led to powders with high crystallinity degree, low water solubility and high bulk density, while the spray of drug solutions led to more amorphous particles, with higher solubility, lower density and improved aerodynamic behaviour.

The optimisation of the operative parameters produced enhanced aerosol performance of the flavonoid powders containing only the active compound.

1. Introduction

The lung, with its large surface and blood supply, is continually exposed to an environment rich in oxygen, and therefore susceptible to injury mediated by oxidative stress. In order to prevent tissue damage, the airways of the lungs are endowed with several antioxidant defences, including glutathione, heme oxygenase, superoxide dismutase, vitamins C and E, beta-carotene, and uric acid [1]. However, when the presence of reactive oxygen or nitrogen species (ROS and RNS, respectively), generated endogenously or exogenously, overcomes the physiologic antioxidant defences, an oxidative stress status occurs causing a number of lung disorders.

Several previous reports have supported the theory of oxidative injury in asthma [2–5], chronic obstructive pulmonary disease (COPD) [6–9], and cystic fibrosis [10], and of a direct damage to epithelial cells [11] by ROS. Hydrogen peroxide and nitric oxide in the exhaled-breath condensates have been used as markers for oxidative processes affecting the lung [12–14]. Thus, the enhancement of the body antioxidant defences through the diet or through a pharmacological treatment can be a proper approach in airways diseases. Among the natural antioxidants, such as glutathione, vitamins, beta-carotene, and selenium, great attention has been focused on flavonoids [15,16], polyphenolic compounds largely present in fruits and vegetables, having anti-inflammatory, antibacterial activities and well-documented antioxidant effect. As a drawback, flavonoids show a poor bioavailability due to their susceptibility to oxidation, degradation in the gastric pH, very slight solubility in water and very low dissolution rate, leading to an irregular absorption from oral solid dosage forms [17]. Although a number of publications have been focused on the antioxidant effect of flavonoids, almost no attention has been addressed to their formulation in order to improve the bioavailability. Recently, oral hydrophilic swellable matrices [18,19] and gastroresistant microparticles have been formulated [20,21]. An alternative strategy may be to achieve higher local concentration of the antioxidant in the respiratory tissue by delivering the aerosol directly to the airways of the lung by a nebulizer, a pressurised metered dose inhaler (pMDI) or a dry powder inhaler (DPI). Compared to nebulisers, DPIs are handy, easy to use and less expensive; moreover, contrarily to pMDI, they are propellant-free and do not require coordination between the device actuation and the patient inspiration. Finally, the dry powder formulation can improve the drug physicochemical and microbiological stability [22], and can assure a higher drug concentration at the deposition site. Thus, the aim of

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the present work was to develop excipient-free dry powders of naringin with aerodynamic properties suitable for inhalation therapy using spray-drying.

Naringin is a flavonoid extracted from grapefruits and citrus fruits and is present in various herbal medicinal formulations marketed in Europe. It was selected as a model of flavonoid because of its interesting anti-inflammatory, anti-cancer and antioxidant properties [23,24].

It is well known that the small particle size necessary to achieve the lung airways deposition (i.e. 1–5 μm) may cause aggregation and poor powder flowability during processing. A number of attempts to improve aerosol performance of the powders, including the modification of density, particle morphology, surface and porosity, as well as the blending of the active drug powder with inert carriers, have been reported [25]. Spray-drying, a one-step process widely used in the pharmaceutical industry to form solid particles from a solution, suspension or emulsion [26], offers an alternative approach to the generation of respirable particles, modifying particle size, morphology and powder density. In order to obtain excipient-free dry powders of naringin with suitable physical and aerodynamic properties from commercially available crystalline material, we studied the influence of the feed compositions and operative conditions on the aerodynamic behaviour and physicochemical characteristics in a series of powders manufactured by spray-drying.

2. Materials and methods

2.1. Materials

Naringin, SPAN 85 and sodium Hydroxide anhydrous pellets were supplied by Sigma–Aldrich (Milan, Italy). Ethanol 96% (for analysis, USP grade), dichloromethane (for analysis, USP grade), *n*-hexane (for analysis, Ph Eur grade) were purchased from Carlo Erba Reagents (Milan, Italy). Size 2 gelatine capsules were kindly offered by Qualicaps Europe S.A. (Madrid, Spain). The device used for respirability tests was the Turbospin® kindly donated by PH&T SpA (Milan, Italy).

2.2. Methods

2.2.1. Powders preparation

Micronised particles were prepared by spray-drying the neat naringin (2–5% w/v) from different solvent feeds, moving from pure water up to a 1:1 v/v water–ethanol ratio (Table 1). In the case of the aqueous feed, naringin (2–3% w/v) was suspended in 200 ml of distilled water under continuous stirring. In the case of water–alcohol mixture feeds, naringin (2–5% w/v) was dissolved in ethyl alcohol (from 20 to 100 ml), and distilled water (from 100 to 180 ml) was added, under continuous stirring, to the resulting solution. In both cases, the suspensions or solutions obtained were sonicated for 10 min, and NaOH 1 M was used to adjust the pH to 7.0 ± 0.1. These liquid feeds were spray-dried using a Buchi mini spray-dryer B-191 (Buchi Laboratoriums-Tecnik, Flawil, Switzerland) under the following experimental conditions: inlet temperature 110 °C, outlet temperature 68–72 °C, feed rate 5 ml/min; nozzle diameter 0.5 mm, drying air flow 500 l/h, air pressure 6 atmospheres, aspirator 100%. Each preparation was carried out in triplicate. The spray-dried powders were collected and stored under vacuum for 48 h at room temperature. The yield of the process was calculated for each batch.

2.2.2. Assessment of the physicochemical properties of powders

The particle size and size distribution of both the raw material and the spray-dried microparticles were obtained with a laser light-scattering granulometer equipped with a micro liquid module (LS 13 320 Beckman Coulter Inc., Fl, USA) and were confirmed by SEM and FM measurements. The LS 13 320 uses a 5 mW laser diode with a wavelength of 750 nm and reverse Fourier optics incorporated in a fiber optic spatial filter systems and binocular lens systems. During preliminary studies, dichlorometane was chosen among other chemicals as the suspending medium. Acidic water and ethyl acetate, in fact, caused the partial solubilisation of the naringin powders according to the reduction in obscuration during the analysis, while chloroform did not allow a good dispersion of the powder even after sonication. The powders were suspended into dichloromethane and sonicated for 2 min: few drops of each sample were poured into the small-volume cell to obtain an obscuration between 8% and 12%. The particle size distributions were calculated by instrument software, using the Fraunhofer model. The analyses were carried out in triplicate for each sample. The results were expressed as $d10$, $d50$, and $d90$, indicating the volume diameters at the 10th, 50th and 90th percentiles, of the particle size distribution and the span is defined as $[d(90) - d(10)]/d(50)$.

Scanning electron microscopy (SEM) was performed on microparticles coated with Au/Pd, using a LEO 420 (LEO Electron Microscopy Ltd., United Kingdom) operating at 14 kV. For the fluorescent microscopy decay measurements, a Zeiss Axiophot fluorescence microscope was used, geared with a 63 × 1.4 NA plan Apochromat oil-immersion objective (Carl Zeiss Vision, München–Hallbergmoos, Germany) and standard DAPI (4′,6-diamidino-2-phenylindole) optics, absorbing violet radiation (max 372 nm) and emitting blue fluorescence (max 456 nm).

Table 1
Feed solutions composition, outlet temperature, yield of the spray drying process and particle size distribution ($n = 3$).

Code #	Feed composition H_2O/EtOH (v/v)	Naringin concentration (% w/v)	Outlet °C	Spray drying yield %	Particle size distribution (μm)			
					$^{d}v10$	$^{d}_{v}50$	$^{d}v90$	Span
#1	100:0	2	72–74	45.9 (10.2)	1.69	5.62	24.7	4.09
#2	9:1	2	70–72	55.6 (6.2)	1.16	3.43	9.25	2.36
#3	8:2	2	69–72	59.3 (5.2)	1.56	5.80	10.5	1.53
#4	7:3	2	64–68	59.4 (0.3)	1.40	5.20	10.1	1.56
#5	6:4	2	71–73	61.2 (0.0)	1.13	4.10	8.42	1.68
#6	1:1	2	72–74	55.9 (1.6)	1.69	6.99	10.1	1.71
#7	100:0	3	63–64	53.6 (1.9)	1.74	6.58	25.1	3.56
#8	9:1	3	64–66	59.2 (9.5)	1.15	3.82	12.9	3.08
#9	8:2	3	70–72	59.4 (9.5)	1.42	5.86	11.2	1.68
#10	7:3	3	67–68	64.4 (2.6)	1.40	5.14	9.81	1.64
#11	6:4	3	64–65	60.8 (5.7)	1.17	4.46	9.47	1.86
#12	1:1	3	68–71	59.3 (2.7)	1.27	4.47	8.47	1.61
#13	1:1	4	72–74	66.5 (10.2)	1.56	6.34	11.1	1.50
#14	1:1	5	70–72	59.7 (13.4)	1.43	5.78	10.4	1.55

Thermogravimetric analyses (TG 50 driven by STARe software, Mettler Toledo, OH, USA) were performed in order to determine the amount of residual solvent (water and ethanol) in spray-dried powders. Each sample was placed in a 100 μL porcelain pan with a pierced cover and heated between 30 and 140 °C at 10 °C/min scan rate, under dynamic flow of dry nitrogen of 200 mL/min. All measurements of the particle size were compared to those obtained by laser-diffraction analyses.

Differential scanning calorimetry (DSC) was performed with an indium-calibrated Mettler Toledo DSC 822e (Mettler Toledo, OH, USA). Accurately weighed samples (3–5 mg) (MTS Mettler Toledo micorbalance, OH, USA) were placed in a 40 μL aluminium pan, which was sealed, pierced and exposed to two thermal cycles as reported elsewhere [27]. For the dehydration cycle measurements, the sample was heated up to 130 °C with a heating rate of 20 °C/min and the temperature was maintained at 130 °C for 15 min in order to remove the residual solvent. Afterwards, the samples were cooled at 25 °C and heated up to 350 °C with a heating rate of 10 °C/min. From this second thermal cycle, the glass transition temperature (T_g), the melting temperature (T_m) and the heat of fusion (ΔH_m) were measured for each spray-dried sample and compared with the corresponding data for the naringin raw material.

The tap density of the spray-dried powders was measured by modifying a method described elsewhere [28]. Briefly, particles were loaded into a bottom-sealed 1 mL plastic syringe (Terumo Europe, Leuven, Belgium) capped with laboratory film (Parafilm® "M", Pechiney Plastic Packaging, Chicago, IL, USA) and tapped on a hard bench until no change in the volume of the powder was observed. The tap density was then calculated from the difference between the weight of the plastic syringe before and after loading, divided by the volume of the powder in the syringe after tapping. The test was carried out in triplicate for each sample.

2.2.3. Aerodynamic behaviour evaluation

The *in vitro* deposition of the micronised naringin was evaluated using a single-stage glass impinger (SSGI, apparatus A European Pharmacopoeia 6.0, Copley Scientific Ltd., Nottingham, UK). A water/ethanol 9/1 v/v mixture was introduced in the upper (7 mL) and lower (30 mL) stages of the SSGI. Hard gelatine capsules (size 2) were filled manually with 20.0 ± 0.5 mg of naringin raw material and spray-dried powders. The capsule was introduced into the Turbospin® and pierced twice. The vacuum pump was operated at a flow rate of 60 L/min for 5 s (Erweka vacuum pump VP 1000 equipped with an electronic digital flowmeter type DFM, Erweka Italia, Seveso (MI), Italy). Each deposition experiment was conducted on 10 capsules and repeated in triplicate.

The upper and the lower stages were washed with 500 ml of a 90/10 v/v water/ethanol mixture. In order to quantify the naringin deposited into the upper and the lower stages of the impinger, UV detection (UV/Vis spectrometer Lambda 25, Perkin-Elmer instruments, MA, USA) was performed at a wavelength of 283 nm, using 1mm spectroscopy SUPRASIL® quartz cell (Hellma 100-QS, HELLMA Italia srl, Milan, I). The analytic method was validated using standard solutions of naringin in the range of 70–400 mg/l ($A = 0.0027c + 0.0147$; $R^2 = 0.9995$). The emitted dose (ED) was gravimetrically determined and expressed as the percentage of the powder exiting the device vs the amount of powder introduced into the capsule. The ratio between the naringin recovered from the SSGI and the naringin emitted from the device determines the recovered dose. The fine particle fraction (FPF), defined as the ratio of the narinigin recovered from the lower stage of SSGI vs the total loaded dose, was expressed as a percentage.

In order to confirm the respirability data obtained by the SSGI, erogation experiments were conducted using an Andersen cascade impactor (apparatus D, European Pharmacopoeia 6.0, ACI, Copley Scientific Ltd., Nottingham, UK) with a modified configuration for the use at a flow rate of 60 L/min as described elsewhere [25,29]. The revised effective cut-off diameters (ECDs) of each stage of the impactor at the higher flow rate were calculated according to [30]:

$$D_{50,1} = D_{50,ref}\left[\frac{Q_{ref}}{Q_1}\right]^{1/2}, \quad (1)$$

where the stage cut-off diameter $D_{50,1}$ at a flow rate Q_1 is related to the stage cut-off diameter ($D_{50,ref}$) at a reference flow rate (Q_{ref}), using the ECD at 28.3 l/min as a reference. Obtained cut-off values are listed herein as stage # followed by the cut-off diameter (μm): −1 (6.87); 0 (6.18); 1 (3.98); 2 (3.23); 3 (2.27); 4 (1.44); 5 (0.76); 6 (0.48); 7 (0.27).

In order to minimize particle bounce, the metal impaction plates were dipped into an *n*-hexane solution of SPAN 85 (0.1% w/v), and the solvent was allowed to evaporate to leave a thin film of SPAN 85 on the plate surface. A filter paper was placed on the filter, the ACI was assembled and the Turbospin® was fitted into a rubber mouth piece attached to the throat. The vacuum pump was actuated for 4 s. The powder deposited on the different stages was recovered by plunging each plate and the stage below into a water/ethanol mixture 90/10 v/v (25–100 ml depending on the stage number). The naringin content was assessed by UV measurements. The emitted dose (ED) and the recovered dose were determined as described above for SSGI experiments. The cumulative mass of powder with a diameter lower than the stated size of each stage was calculated and plotted as a percentage of powder recovered against the cut-off diameter. The mass median aerodynamic diameter (MMAD) of the particles was extrapolated from the graph, according to the European Pharmacopoeia 6.0. From the same plot, the fine particle dose (FPD), i.e. the mass of the active ingredient with a particle size less than 5 μm, and the fine particle fraction (FPF), i.e. the fraction of powder emitted from the device with a particle size less than 5 μm, were determined.

2.2.4. Dissolution study

The analysis of the powder solubility was impossible to carry out because of the tendency of the amorphous spray-dried powders to rapidly revert to the crystalline state after exposure to the solvent. Information about the improvements in the immediate solubility of the spray-dried powders was obtained by a modified dissolution study [31]. Briefly, an excess of micronised powder (1.6 g) was introduced along with a magnetic bar into a 40 ml closed, flat-bottomed glass vial, and 25 ml of distilled water heated at 37 °C were added. The dissolution medium was kept at 37 °C in a water bath under magnetic stirring at 300 rpm: at regular intervals, the liquid phase was withdrawn, replaced with distilled water at the same temperature, filtered with 0.45 μm filters, diluted and was analysed by UV-visible spectroscopy for naringin content. Dissolution tests were conducted in triplicate, and the observation protracted for 120 min. Results are reported at 30 min. After each dissolution study, the solid material precipitated was recovered by filtration, dried under nitrogen and analysed by DSC in order to confirm its crystalline state.

3. Results and discussion

3.1. Manufacturing and characterisation of spray-dried powders

Different excipient-free dry powders for pulmonary delivery of naringin were prepared with the aim of studying the influence of the drug concentration, ethanol content in the feed solutions and operative conditions of the spray-drying process on the physicochemical and aerodynamic properties of the obtained product.

Table 1 presents all the manufactured powders, containing 2% (batches #1–#6), 3% (batches #7–#12), 4 and 5% w/v of naringin (batches #13 and #14, respectively). Water and water/ethanol mixtures ranging from 9:1 to 1:1 v/v were used for the feed preparation.

The commercially available naringin (N) was a crystalline powder (Fig. 1), featuring needle-shaped crystals, with a very slight water solubility (1.1 g/l) [20]. By increasing the proportion of ethanol in the feed (Table 1), so as to favour the solubility of the drug, we observed that the feeds turned from suspensions (#1–#3, #7–#10) into true solutions (#4–6 and #11–#14) and that the process yield was improved. The use of ethanol/water 1:1 v/v allowed the increase of drug concentration from 2% w/v to 5% w/v in the feed, maintaining acceptable yields (over 55%).

The thermal gravimetric analyses exhibited no significant differences in the residual solvent content, ranging between 5.5% and 6.5% for each sample.

Concerning the particle size and morphology, the powders (batches #1, 2% N and #7, 3% N) from the 100% water feeds presented a median volume diameter (d_{50}) of 5.62 and 6.58 μm and the highest values of d_{90} (24.7 and 25.1 μm) (Table 1) and tapped density (Fig. 2). Moreover, the particles showed a high degree of crystallinity at SEM analyses (Fig. 3 a). The addition of ethanol into the solvent feed led to a sensible reduction of d_{90} and span, as observed in batches #2 and #8 (Table 1). The very high span values recorded for batches #1 and #7 suggest a broad particle size distribution ascribable to the concomitant presence of crystals and amorphous particles, observed by microscopy. It is well known that the laser diffraction technique, modelling the particles as spheres, leads to a heterogeneous particle distribution for powders containing both crystalline and amorphous material (#1, #2, #7 and #8). Microscopy observation revealed that powders in batches #2 and #8 presented much thinner crystals (Fig. 3 b and c) than those in batches #1 and #7; this would account for the reduction in median diameter values (Table 1). Some of the particles were amorphous, bigger and pierced (Fig. 3b and c), featuring holes that maybe caused by crystals during the spray-drying process. As a result of this characteristic morphology, the powders that were dried from 10% ethanol feeds in both naringin concentrations (2–3% w/v) showed the lowest tapped density (Fig. 2). The powders (2–3% N w/v) prepared from 20% to 40% of ethanol feeds showed a decrease in the particle size (Table 1) accompanied by the disappearance of residual crystals and the formation of smaller, corrugated amorphous particles, shown by the scanning electron microphotographs (Fig. 3d). Finally, powders from water/ethanol 1:1 v/v feed showed fine particles with undefined shape, typical of amorphous powders. Batch #6 (2% N) was made of collapsed particles, aggregated to each other and was difficult to process (Fig. 3e). An increase in naringin concentration led to slightly bigger particles, still collapsed but better separated, as in batches #12 (Fig. 3f) and #14.

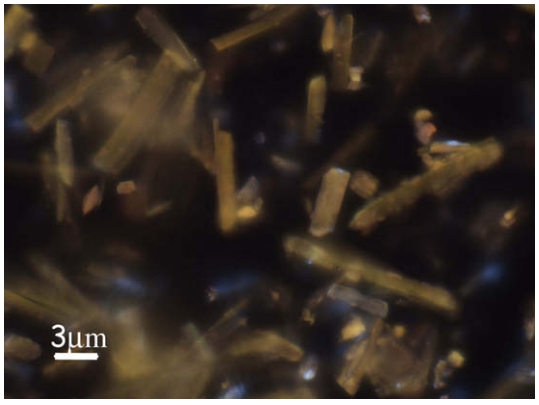

Fig. 1. FM picture of Naringin raw material.

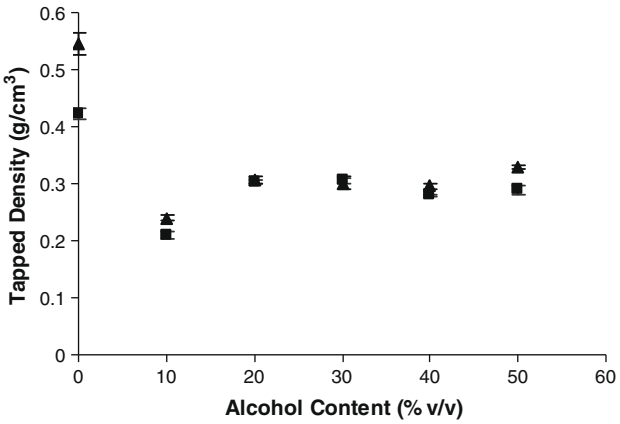

Fig. 2. Tapped density versus alcohol concentration in the spray feeds. (■) naringin 2% w/v and (▲) naringin 3% w/v.

In accordance with the microscopy observations, the DSC profiles of spray-dried powders (Fig. 4) confirmed the partial (batch #1, line b) or total (batch #6, line c) loss of the crystalline state of naringin, as a result of the increased ethanol content in the feeds. As reported in the literature [20], the thermogram of N in the crystalline state (Fig. 4, line a) showed the phase transition peak at 161.46 °C followed by a peak at 174.96 °C, probably due to molecular rearrangement. The final endothermic melting peak was at 246.96 °C with peaks occurring over 250 °C attributed to naringin decomposition. The thermogram of naringin powders formulated by spray-drying the aqueous suspension (batch #1, Fig. 4, line b) shows both the peaks of phase transition at 161.46 °C and melting point at 247.37 °C with smaller intensity. The thermograms of the powders manufactured from feeds with higher ethanol content (batches #6, #12, #13 and #14; Figs. 4 and 5) only show the peak related to the phase transition of naringin: the absence of the endotherm corresponding to the crystal melting point may be interpreted as a consequence of amorphisation. DSC results were in agreement with X-ray assessments, showing no sign of crystallinity in the batches obtained from feeds with ethanol ratios higher than 30% v/v (data not shown).

These results may be explained by the formation of amorphous material in the solutions where the higher ethanol content is able to increase the powder solubility.

To confirm this hypothesis, the dissolution profiles in distilled water of all spray-dried powders, obtained by the modified method mentioned above [31], were compared with those of crystalline raw material (Figs. 6 and 7, semi-logarithmic scale). The results showed a greater immediate solubility of all powders produced from alcoholic feeds (Fig. 6) and the occurrence of the peak solubility of the amorphous dry powders (batches #6, #12–#14) within the first 5 min of the analyses, with values up to 50 times higher than for the naringin raw material. In all cases, the solubility started declining very quickly, reaching a nearly constant value after 40–60 min, due to the crystallisation of the amorphous spray-dried powders suggested by DSC analysis of the precipitated material (data not shown).

3.2. Aerodynamic behavior

Firstly, the *in vitro* deposition of the dry powders using Turbospin® as inhaler device was tested by a single-stage glass impinger. The emitted dose obtained from all spray-dried powders was higher than 80%, with the exception of the powders spray-dried from 4

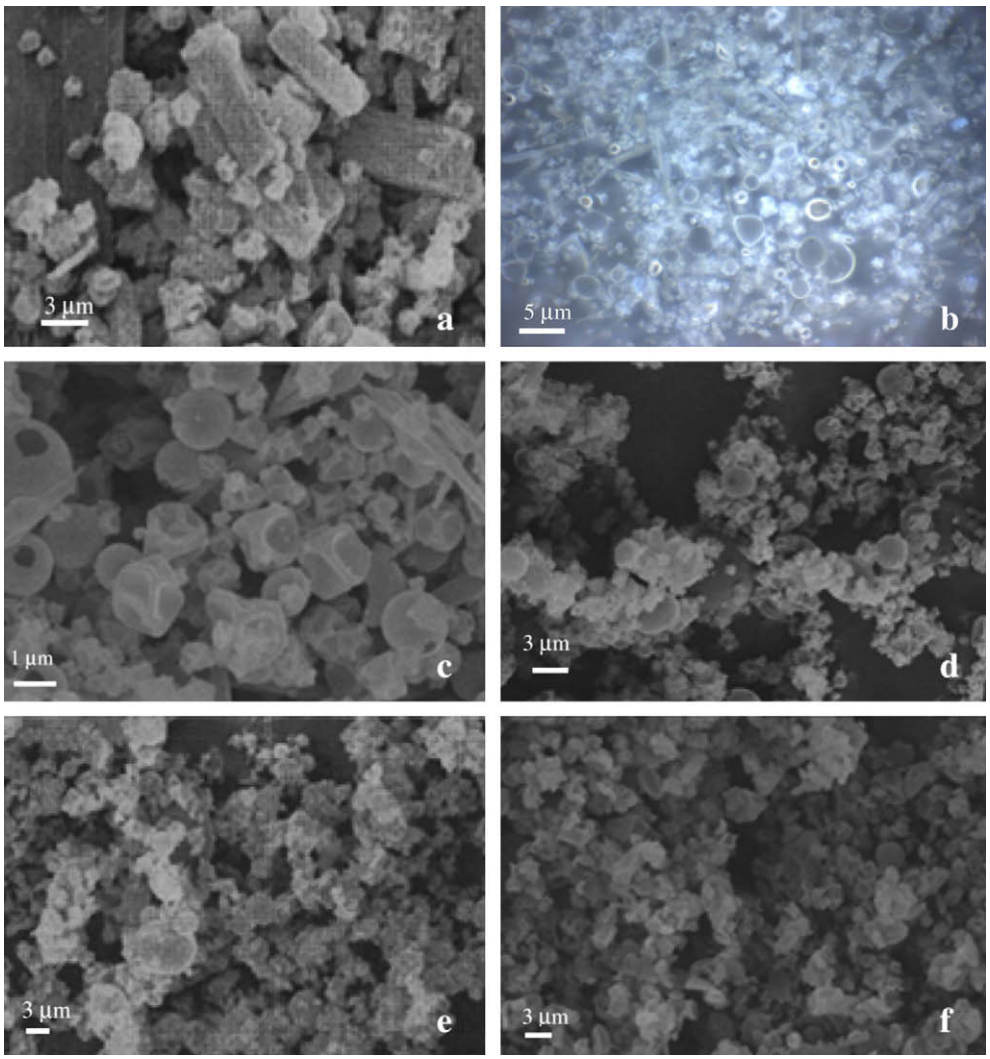

Fig. 3. SEM picture of (a) batch #1 – 2% w/v naringin water suspension; (c) batch #2 – water/ethanol ratio 9:1 v/v and naringin 2% w/v; (d) batch #4 – water/ethanol ratio 7:3 v/v and naringin 2% w/v; (e) batch #6 – water/ethanol ratio 1:1 v/v and naringin 2% w/v; (f) batch #12 – water/ethanol ratio 1:1 v/v and naringin 3% w/v; (b) FM picture of batch #2 – water/ethanol ratio 9:1 v/v and naringin 2% w/v.

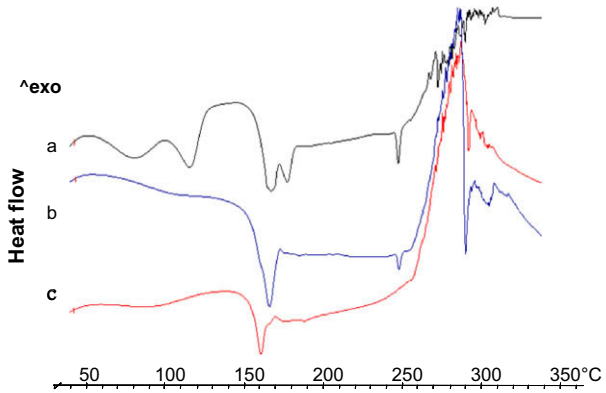

Fig. 4. Differential scanning calorimetry thermograms of naringin raw material (a), batch #1 (b), batch #6 (c).

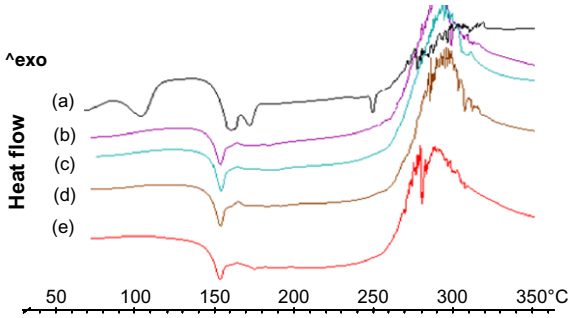

Fig. 5. Differential scanning calorimetry thermograms of naringin raw material (a), batch #6 (b), batch #12 (c), batch #13 (d) and batch #14 (e).

and 5% w/v naringin feeds (batches #13 and #14). The recovered dose was higher than 80% in all cases (data not shown). The fine particle fractions (FPF) were between 21.2% and 44.5%, depending on the feed composition (Table 2). By plotting alcohol concentration in the feeds *versus* FPF (Fig. 8), it is possible to observe, as also previously reported [25], that the aerodynamic properties of the powders were influenced by the ethanol content of the liquid feed and the solubility of the drugs. For naringin, a very slightly soluble drug, the maximum FPF (44.5%) was obtained for powders manufactured with 30% alcohol and 2% drug in the feed (batch #4). As the drug concentration increased to 3% (batches #11 and #12), an increased quantity of alcohol (40–50%) was necessary in order to have similar FPF values (41.7 and 42.5, respectively) (Fig. 8).

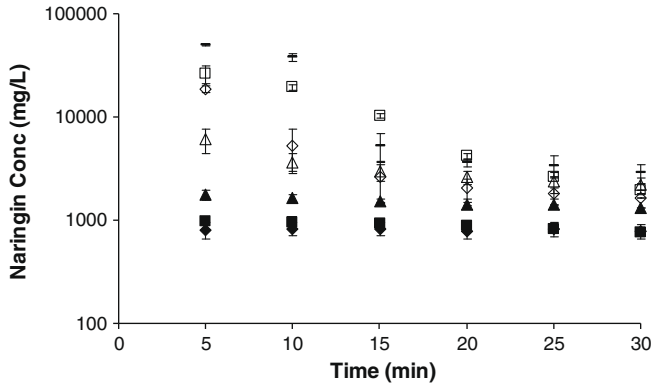

Fig. 6. Aqueous solubility at 37 °C for naringin raw material and spray-dried powders from different feed solutions (2% naringin w/v): (■) Naringin raw material; (♦) batch #1; (▲) batch #2; (Δ) batch #3; (◇) batch #4; (-) batch #5 and (□) batch #6.

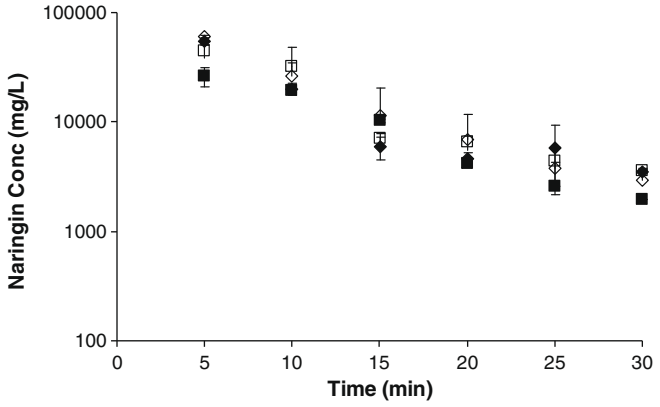

Fig. 7. Aqueous solubility at 37 °C for spray-dried powders with increasing naringin concentration up to 5% w/v (1:1 water/ethanol ratio): (■) batch # 6; (♦) batch #12; (◇) batch #13 and (□) batch #14.

Table 2
Emitted dose, FPF, MMAD and FPD of naringin spray-dried powders after single-stage glass impinger and Andersen cascade impactor deposition experiments.

Code #	Single Stage Glass Impinger		Andersen Cascade Impactor		
	ED (%)	FPF (%)	MMAD (μm)	FPD (mg)	FPF (%)
#1	89.6 (5.24)	22.6 (2.73)	3.45	6.39	31.9
#2	91.5 (0.16)	35.8 (2.05)	3.54	8.74	44.3
#3	82.5 (4.59)	35.0 (0.41)	3.48	10.2	51.4
#4	92.7 (0.02)	44.5 (1.54)	3.41	9.76	48.7
#5	84.2 (6.48)	40.9 (1.36)	3.78	10.1	50.6
#6	83.9 (2.35)	38.5 (0.06)	3.41	9.81	48.5
#7	89.6 (2.12)	21.2(2.12)	3.92	5.02	25.2
#8	90.4 (4.17)	34.5 (1.77)	3.57	9.23	46.6
#9	87.1 (1.46)	38.4 (0.25)	3.40	9.62	47.9
#10	74.9 (1.98)	35.0 (0.30)	3.50	9.82	49.1
#11	83.3 (2.19)	41.7 (4.88)	3.29	11.1	55.8
#12	84.1 (2.23)	42.5 (1.31)	3.41	8.60	42.9
#13	74.7 (8.77)	34.3(4.11)	3.34	7.70	38.7
#14	77.1 (3.32)	33.3 (1.56)	3.69	6.90	35.0

ED, Emitted dose; FPF, Fine particle fraction; MMAD, Mass median aerodynamic diameter; FPD, Fine particle dose.

At further increase of the naringin concentration to 4% and 5% in the 50% ethanol feed solutions (batches #13 and #14), a worsening of the aerodynamic properties of the powders (FPF 34.3 and 33.3, respectively, Table 2) was observed.

The results related to the aerodynamic behavior of the powders, obtained with the SSGI, were compared with the results obtained

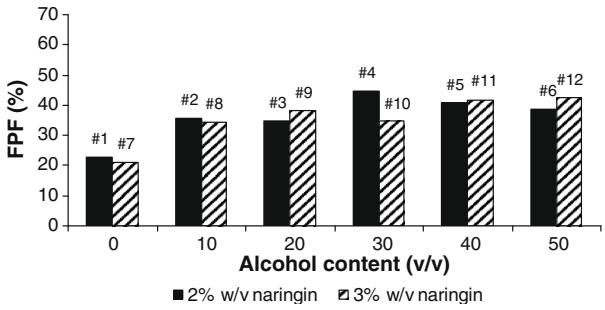

Fig. 8. Fine particle fraction versus alcohol concentration after single-stage glass impinger deposition experiments.

from the deposition experiment conducted with the Andersen cascade impactor (ACI). The recovered dose was ≈80%, i.e., within 75% and 125%, in accordance with European Pharmacopoeia 4th edition (data not shown). Figs. 9 and 10 show the amount of drug deposited on the throat, on the stages 1–7, and on the filter, expressed as a percentage of the totality of powder recovered. The powder manufactured from the water feed (#1, Fig. 9) exhibited a massive deposition into the throat (≈40%). The increase of ethanol content in the feed solution led to powders with reduced impact into the throat (≈20%) and increased impact in the lowest stages (Fig. 9), corresponding *in vivo* to the trachea and the deeper airways [32]. Generally, the best aerodynamic behavior was observed for batches #3 and #5 (Fig. 9) manufactured with 20% and 40% ethanol, respectively, in the feed (Table 2). Similar ACI deposition patterns were observed for the batches prepared by increasing the naringin concentration in the feed from 2% to 3% (data not shown). On the contrary, further increases in the naringin concentration (4% or 5% w/v) caused an increased deposition into the throat (Fig. 10), in accordance with the low FPF values obtained from SSGI deposition experiments.

Also, the mass median aerodynamic diameter (MMAD) along with the fine-particle dose (FPD) and the fine-particle fraction (FPF), summarised in table 2, indicated that the powders spray-dried from water (#1 and #7) presented the worst aerodynamic properties (FPD 6.39 and 5.02; FPF 31.9% and 25.2%, respectively). This behaviour can be explained as a consequence of the presence of large crystals in the powders, which caused a significant deposition on the upper stages of the ACI. The reduction in density of powders #2 and #8 was caused by the presence of pierced particles and thinner crystals, as observed by microscopy, and corresponded to a slight improvement of FPF (44.3% and 46.6%) and FPD (8.74 and 9.32). This behaviour may be explained with the presence of agglomerates formed by little spheres stacked to the crystals that counteract the particles ability to fly. Finally, the increase of ethanol content up to 40% in the feed liquids caused a quick improvement in FPF values to 50.6 (batch #5) and 55.8% (batch #11). Powders from feed with 50% ethanol content showed a worsening of the aerodynamic properties, confirming the results from SSGI analyses. These powders (#6, 12–14, Table 2) seem to be cohesive and, during the aerosolisation, they flow as aggregates rather than discrete particles, thus impacting on the upper ACI stages. In the case of powders with naringin ratio in the feed of 4% and 5% (batches #13 and #14), the lower FPF and FPD values were also due to a larger geometric diameter (Table 1).

4. Conclusion

Naringin is a potent free-radical scavenger drug capable of enhancing antioxidant defences of the lung in patients with airways

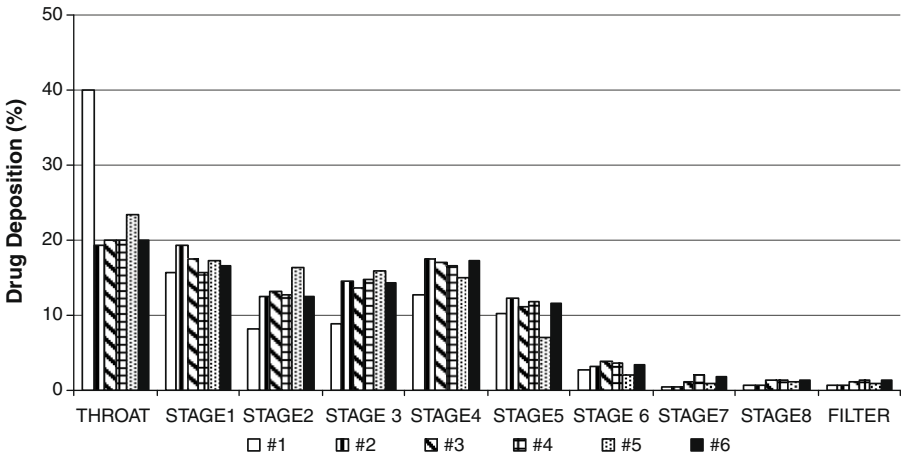

Fig. 9. Andersen cascade impactor deposition pattern after the aerosolization of powders dried from feeds containing 2% w/v of naringin and different water/ethanol ratios: #1, 100:0; #2 9:1; #3, 8:2; #4 7:3; #5, 6:4; #6 1:1.

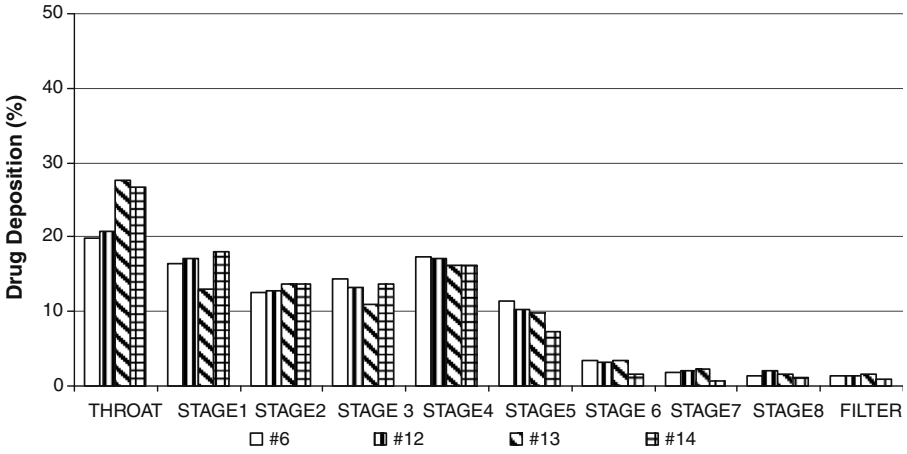

Fig. 10. The Andersen cascade impactor deposition pattern after the aerosolization of powders dried from feeds with a water/ethanol ratio of 1:1 v/v and different naringin concentrations #6, 2%; #12, 3%; #13, 4%; #14, 5%.

diseases. Like other flavonoids, it is irregularly and poorly absorbed from oral dosage form due to its pharmacokinetics characteristics. Hence, DPI systems, directly delivering naringin powder to the airways of the lung, may be able to achieve a high local concentration of the antioxidant molecule and enhance its physicochemical and microbiological stability, thus improving the therapeutic effect. The present research shows that a fine excipient and propellant-free powder with satisfactory aerodynamic behaviour can be produced by spray-drying solutions of pure naringin, carefully selecting the appropriate drug concentration and water/ethanol feed ratio. Spray-drying seems to provide a simple means to obtain naringin amorphous powders with controlled shape, respirable size, appropriate tap density and increased immediate solubility, useful for airways deposition.

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