Drug Development and Industrial Pharmacy, 2011; 37(11): 1376–1386

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.576680

informa
healthcare

RESEARCH ARTICLE

Preparation and evaluation of poly(lactic-*co*-glycolic acid) microparticles as a carrier for pulmonary delivery of recombinant human interleukin-2: II. *In vitro* studies on aerodynamic properties of dry powder inhaler formulations

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Abstract

Objective: The aim of this study was the preparation and evaluation of dry powder formulations of recombinant human interleukin-2 (rhIL-2)-loaded microparticles to be administered to the lung by inhalation.

Methods: As indicated in our previous study, the microparticles were prepared by modified water-in-oil-in-water ($w_1/o/w_3$) double emulsion solvent extraction method using poly(lactic-*co*-glycolic acid) (PLGA) polymers. The dry powder formulations were prepared with blending of microparticles and mannitol as a coarse carrier. The actual aerodynamic characteristics of the microparticles alone and prepared mixtures with mannitol are evaluated by using the eight-stage Andersen cascade impactor.

Results: Due to the low tapped density of microparticles (<0.4 g/cm^3), the theoretical aerodynamic diameter (MMADt) values were calculated (<5 μm) on the basis of the geometrical particle diameter and tapped density values. The lowest tapped density value (0.17 g/cm^3) belongs to the cyclodextrin-containing formulation. According to the results obtained using the cascade impactor, the emitted doses for all microparticle formulations were found to be rather high and during the aerosolization for all the formulations except F3 and F5, >90% of the capsule content was determined to be released. However, the actual aerodynamic diameter (MMADa) values were seen to be higher than the MMADt values. The blending of the microparticles with mannitol allowed their aerodynamic diameters to decrease and their fine particle fraction values to increase.

Conclusion: The obtained results have shown that the mixing of rhIL-2-loaded microparticles with mannitol possess suitable aerodynamic characteristics to be administered to the lungs by inhalation.

Keywords: Dry powder inhalation, pulmonary drug delivery, coarse carrier, mannitol, interleukin-2, PLGA microparticles

Introduction

Lung cancer is the leading cause of cancer-related deaths and accounts for more deaths than colorectal, breast, prostate, and pancreatic cancers combined. Presently available treatment options of lung cancer include surgery, radiation therapy, and chemotherapy, either alone or in combination, depending on the stage of the cancer[1]. However, radiotherapy causes damage to normal cells around cancer cells and chemotherapy needs high-dose level anticancer drugs to treat cancer. From these, both of them have dose-limiting toxicity[2].

The occurrence of a large number of the tumors in immunocompromised patients indicates a role of the immune system in the control of tumor growth. The possibility that cancers can be eradicated by specific immune responses has been the impetus for a large body of work in the field of tumor immunology.

Interleukin-2 (IL-2), which is one of the cloned cytokines, has many immunologic effects including an increase in T-cell proliferation, T-cell activation, monocyte and natural killer cell activation[3]. Due to the potent immunomodulatory effect of IL-2, recombinant human

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(Received 27 January 2011; revised 24 March 2011; accepted 27 March 2011)

IL-2 (rhIL-2) was tested in the treatment of tumors in clinical studies[4,5] and was approved for the treatment of patients with metastatic renal cell carcinoma and metastatic melanoma by the United States of America Food and Drug Administration (FDA). On the other hand, the *in vivo* half-life of rhIL-2 is very short ($t_{1/2}$ = 13 min) and for this reason frequent administration of high-dose rhIL-2 is required to obtain a therapeutic effect[6]. The primary limitation with the use of high-dose rhIL-2 therapy is the associated toxicity. Serious cardiovascular, renal, hepatic side effects and most importantly life-threatening reactions like capillary leak syndrome have developed after intravenous (i.v.) administration[7]. Local administration of rhIL-2 was considered as an approach to overcome these side effects caused by high doses of systemic rhIL-2. Local administration of rhIL-2 minimized systemic effects while obtaining a high rhIL-2 concentration in the action site[8]. T cells, which have a critical role for an effective antitumor response, are located mainly at the tumor region. For this reason, rhIL-2 level at the site of the tumor, which is responsible for T-cell proliferation and activation is very important[9]. A greater beneficial effect could be expected after local IL-2 application such as peritumoral, intratumoral, intra-arterial, intracavitary, or inhalation[8].

Inhalation route is an effective method to deliver therapeutic agents to the respiratory tract. Local delivery of medication to the lung is highly desirable, especially in patients with specific pulmonary diseases like cystic fibrosis[10], asthma[11], chronic pulmonary infections[12], or lung cancer[13].

Pressurized metered-dose inhalers (pMDI), nebulizers, and dry powder inhalers (DPIs) are three main delivery systems used for aerosol inhalation in humans[14]. Among these, DPI appears to be the most promising for future use[15]. Compared with nebulizers, 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. The dry powder formulation can improve the physicochemical and microbiological stability of the drug[16] and can assure a higher drug concentration at the deposition site. In addition, they have a long history of successful use in the treatment of both local and systemic diseases.

Inhalable powders have to fulfill certain requirements; the increase of the respirable fraction with a decreasing aerodynamic particle diameter is a fundamental matter of fact[17]. The general assumption is that particles with the diameter of 1–5 μm to the specific site of the respiratory tract[18]. However, the small particle size necessary to achieve the lung airways deposition may cause aggregation and poor powder flowability during processing. A number of attempts to improve DPI 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[19].

DPI formulations generally incorporate at least one other component, as a carrier to facilitate aerosolization of the active agent. Carrier particles are used to improve drug particle flowability, thus improving dosing accuracy and minimizing the dose variability observed with drug formulations alone while making them easier to handle during manufacturing operations[14,20]. With the use of carrier particles, drug particles are emitted from capsules and devices more readily; hence, the inhalation efficiency increases[21]. The large carrier particles normally impinge in the buccal cavity or in the oropharynx, whereas liberated micronized drug particles that overcome the forces of adhesion to the carrier particles deposit further down the respiratory tract[22]. The carrier of choice for DPI products is currently lactose monohydrate[23]. The advantages of lactose are its well-investigated toxicity profile, its broad availability, and its relatively low price. Particles of lactose can also be produced with predetermined properties such as with a smooth surface and good flow properties. However, the use of lactose has some disadvantages, such as its incompatibility with drugs (such as formoterol, budesonide, and peptides) that have primary amine moieties[24]. Moreover, lactose cannot be used in the development of formulations for diabetic patients. Other excipients such as mannitol have been suggested as possible alternative carriers for DPI formulations[23,25]. Mannitol, a hexahydric alcohol, is an attractive alternative carrier to lactose because it does not have a reducing effect, it is less hygroscopic than some of the other sugars, gives a high sweet after-taste, which could be used to confirm to the patient that a dose has been successfully administered, and it has the capacity to provide a high fine particle dose of incorporated drug upon powder aerosolization[26,27].

In our previous study, poly(lactic-*co*-glycolic acid) (PLGA) microparticles containing rhIL-2 were manufactured by modified water-in-oil-in-water ($w_1/o/w_3$) double emulsion solvent extraction method and the effects of various formulation parameters on the physicochemical properties of the microparticles were investigated[28,29]. In this study, *in vitro* aerodynamic properties of the microparticles were investigated by using an eight-stage Andersen cascade impactor (ACI) and their suitability for dry powder inhalation systems were evaluated. Additionally, interactive mixtures of mannitol as a coarse carrier with the microparticles were manufactured and tested for their aerosolization behavior.

Materials and methods

Materials

Recombinant human interleukin-2 (rhIL-2) was kindly supplied by Novartis (Turkey). When the white lyophilized powder is reconstituted with 1.2 mL of water, each vial contains per mL solution: 1 mg (18×10^6 IU of rhIL-2, 50 mg (5% w/v) of mannitol, and 0.2 mg (0.002% w/v) of sodium dodecyl sulfate (SDS), buffered with sodium phosphates to a pH of 7.5 (range 7.2–7.8).

PLGA Resomer® RG 502 (50:50 lactic to glycolic acid ratio and a Mw = 12 kDa, inherent viscosity 0.24 dL/g), Resomer® RG 502H (50:50 lactic to glycolic acid ratio and a Mw = 12 kDa, inherent viscosity 0.20 dL/g), Resomer® RG 752H (75:25 lactic to glycolic acid ratio and a Mw = 11 kDa, inherent viscosity 0.19 dL/g), and Resomer® RG 756S (75:25 lactic to glycolic acid ratio and a Mw = 90 kDa, inherent viscosity 0.77 dL/g) were purchased from Boehringer Ingelheim (Ingelheim, Germany). H signifies PLGA terminated with a carboxylic acid group. Chitosan chloride (Protosan UP CL 113) was obtained from Novamatrix (San Diego, CA). Carbopol 971P NF was obtained from Noveon (Cleveland, OH). Hydroxy-propyl-β-cyclodextrin (HPβCD, Mw = 1.460, molar substitution 0.8), poly(vinyl alcohol) (PVA) (88 mol% hydrolyzed, Mw = 30,000–70,000) and dichloromethane (99.9%, HPLC grade) were from Sigma (Germany). Microbicinchoninic acid (microBCA) protein assay reagent kit was purchased from Pierce Biotechnology (Rockford, IL). Mannitol (Pearlitol® 50C) was kindly supplied by Roquette-Pharma (Germany). The Aerolizer® inhaler (Novartis Pharmaceutical, Turkey) was used as a model inhalation system for the evaluation of aerodynamic characteristics of the formulations. Hard gelatine capsules, number 3, from Capsugel (Belgium) were used. All other chemicals used were of analytical grade. Ultrapure water (Millipore Model Milli-Q Water Purification System, Canada) was used for all experiments.

Preparation of microparticles

A modified water-in-oil-in-water ($w_1/o/w_3$) double emulsion solvent extraction technique was used for microparticle fabrication as described in our previous study[28,29]. In brief, the organic phase was prepared by dissolving PLGA (200 mg) in 1.5 mL of dichloromethane. The organic solution was emulsified with aqueous solution of rhIL-2 (3.6×10^6 IU rhIL-2 in 0.2 mL) by using a high-speed homogenizer equipped with a 10-mm shaft (Ultra Turrax® T-25; Ika, Staufen, Germany) operating at 13,500 rpm for 2 min. At the F4 coded microparticle formulation, the inner aqueous phase composed of solution of rhIL-2: HP-β-CD at a 1:5 molar ratio of protein to cyclodextrin. The resulting primary emulsion (w_1/o) was injected into the external aqueous phase consisting of 60 mL of 10% (w/v) polyvinyl alcohol (PVA) solution and homogenized at 9500 rpm for 5 min to form the secondary emulsion ($w_1/o/w_2$). Then, the obtained $w_1/o/w_2$ emulsion was transferred into 300 mL of 0.5% (w/v) PVA solution (w_3) and stirred with a mechanical stirrer (Eurostar Power Control-Visc 6000; Ika) at 700 rpm for 3 h to extract the organic solvent for subsequent particle hardening at room temperature, which was controlled at 25 ± 2°C. Finally, the microparticles were collected by centrifugation (Sigma 2-16P, Germany) at 5000 rpm for 15 min, washed with ultrapure water (Milli-Q water) three times, and lyophilized to obtain free flowing powder. The dried microparticles were stored in a sealed glass vial and placed in a desiccator at 4°C.

The surface of the PLGA microparticles was modified by adsorption method[29–31]. In brief, during the preparation of PLGA microparticles described above, the primary emulsion (w_1/o) obtained by homogenization was injected to PVA solution containing 0.5% (w/v) chitosan chloride or Carbopol 971P. Other steps were the same as described above.

Compositions used for rhIL-2 loaded microparticles are shown in Table 1.

Morphology of microparticles

The shape and surface morphology of microparticles were investigated by scanning electronic microscopy (SEM) (Jeol Model JSM-6400, Tokyo, Japan). For the sample preparation, a small aliquot of the microparticles were mounted onto metal stubs using double-sided adhesive tape. Excess microparticles were removed by tapping the stub sharply. After being vacuum-coated with a thin layer (100–150 Å) of gold at 25 mA current and 10^{-5} Torr pressure for 200 sec, the microparticles were examined by SEM operated at 15 kV accelerating voltage. The photomicrographs were then taken at a magnification of 5000×.

Particle size analysis of microparticles

Particle size and size distribution of microparticles were measured with a laser diffraction particle size analyzer (Sympatec Helos Model H0849, Germany). In brief, proper amounts of dry microparticles were mixed with distilled water containing 0.1% Tween 20 and suspended completely for several minutes using an ultrasonic bath. The suspension was then placed in the laser particle counter. The size is measured at 25 ± 2°C. Each sample

Table 1. Composition and properties of chosen microparticle formulations.

Formulation code	Type of polymer	Excipients in aqueous inner phase	Coating material	Mean particle size (μm ± SD)	EE (% ± SD)
F1	RG 502	—	—	4.02 ± 0.01	99.22 ± 1.20
F2	RG 502H	—	—	1.57 ± 0.00	92.73 ± 1.00
F3	1:1 RG752H:RG756S	—	—	3.12 ± 0.01	98.15 ± 1.21
F4	RG 502	HP-β-CD	—	3.21 ± 0.01	84.12 ± 1.43
F5	RG 502	—	Chitosan chloride	5.68 ± 0.03	97.54 ± 2.36
F6	RG 502	—	Carbopol 971	1.37 ± 0.01	98.17 ± 1.53

EE (%) = Encapsulation efficiency.
SD = Standard deviation, $n = 3$.

was measured in triplicate. Medium particle size (D_{50}, the particle size when cumulative value is 50% by volume in the particle size cumulative distribution profile) and particle size distribution were measured. The size distribution was evaluated with the span value defined using Equation (1)[32]:

$$\text{Span} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \quad (1)$$

where $D_{N\%}$ (N=10, 50, 90) is the volume percentage of microparticles with diameters up to $D_{N\%}$ is equal to N%. The smaller span value indicates the narrower size distribution.

Preparation of dry powder formulations

The coarse carrier (mannitol) was geometrically blended with rhIL-2-loaded microparticles to provide a final ratio (mannitol:microparticle) of 9:1 w/w. All formulation blends were then stored in tightly sealed glass vials.

Determination of the homogenicity of dry powder formulations

After blending microparticles with mannitol, the homogenicity of the mixture was determined by controlling the drug content in each dry powder formulation. Five randomly selected samples were taken from different positions form the blend and analyzed. Each sample weighing 40 ± 1.0 mg (this was the amount of the powder mixture to be introduced into each capsule) was dissolved in 1 mL of 0.1 M NaOH containing 2.0% (w/v) SDS for 24 h at room temperature. After incubation, the solution was centrifuged (Sigma 2-16P, Germany) at 4000 rpm for 5 min. The supernatant was neutralized with 0.1 M HCl[33]. The amount of the rhIL-2 in each powder formulation was determined using microBCA protein assay reagent kit according to the instructions of the manufacturer. The degree of content uniformity (homogenicity) was expressed in terms of the percentage coefficient of variation (CV%) and if the percentage of CV was <6%, content uniformity was noted as acceptable[34].

Determination of powder density, flowability, and primary aerodynamic diameter

The bulk density of the microparticles alone and dry powder formulations containing the blend of microparticles with mannitol were determined by filling a known mass of powder (100 mg) under gravity into a 5 (±0.05) mL measuring cylinder and recording the volume occupied by the powder. The tapped densities of the powders was determined by tapped density measurements on the same samples using an automatic tapper (Aymes, Turkey) until no further change in the powder volume was observed[22,35]. The preliminary results showed that the use of 1250 taps was sufficient to attain the minimum volume of the powders under study. Measurements were performed in triplicate. The bulk and tapped densities of the powders were calculated by dividing the weight by the corresponding bulk volume or tapped volume recorded.

Carr's index and Hausner ratio values for each powder formulation were calculated from bulk density and tapped density data and employed as an indication of flowability of the samples. Carr's index and Hausner ratio were calculated using the following equations[27]:

$$\text{Carr's index} = \frac{\text{Tapped density-bulk density}}{\text{Tapped density}} \times 100 \quad (2)$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (3)$$

Carr's index values of <25% and Hausner ratio values <1.25 indicate acceptable flow properties.

Theoretical estimates of the particle primary aerodynamic diameter (MMADt) were derived from the particle sizing and tapped density data, according to Equation (4)[36,37]:

$$\text{MMADt} = d(\rho/\rho_0 X)^{1/2}, \quad (4)$$

where d is the geometric mean diameter, ρ_0 is a reference density of 1 g/cm^3, and X is the dynamic shape factor, which is 1 for a sphere.

Capsule filling

Each microparticle formulation alone and mixtures containing microparticles and mannitol were filled manually into hard gelatin capsules (size 3) with the weight of 40.0 ± 1.0 mg.

Aerodynamic characteristics and aerosol performance

The actual aerodynamic characteristics and the aerosol performance of the formulations were tested by eight-stage Mark II ACI according to the European Pharmacopoeia 6.0[38] (Copley Scientific Ltd., Nottingham, UK). The ACI consisted of initiation port (IP) and the pre-separator (PS), seven stages and a final collection filter (Whatman®; pore size, <70 mm). Schematic diagram of ACI components was shown in Figure 1. The pre-separator was attached to the impactor to prevent large particles from aggregating or from reaching rear stages. In order to prevent particles from bouncing off the plates and becoming re-entrained in the air stream, the metal impaction plates were dipped into Tween 20 solution in ethanol (1% v/v), and the ethanol was evaporated in the oven at 60°C for 5 min to leave a thin film of Tween 20 on the plate surface. The ACI was then assembled from the filter stage to stage 0 onto which the pre-separator was placed and the induction port was attached. The ACI was subsequently connected to a vacuum pump (Copley Scientific Ltd.) equipped with an electronic digital flow meter (Copley Model DFM2; Copley Scientific Ltd.). The air flow rate was then adjusted to 60 L/min. A filled capsule was placed into the sample compartment of the Aerolizer®

DPI device attached to the induction port using a rubber mouthpiece adaptor providing good seal. The capsule was pierced as directed in the user instructions and the vacuum pump was switched on. The pump was operated for 5 sec so that a steady flow rate of 60 L/min was achieved, and the dose was released. The pump was operated for another 5 sec at the established flow rate following the release of the dose and it was then switched off. In all cases, eight capsules were discharged into the apparatus per determination and each experiment was repeated in triplicate. After actuation, the ACI was disassembled and each impactor section was individually rinsed thoroughly, recovering any powder deposits, using a collecting solution. The 0.1 M NaOH solution containing 2.0% (w/v) SDS was used as the collecting solution in this study, since this had been used to hydrolyze and dissolve the PLGA in microparticle formulation. After dissolving the powders, the protein concentrations of the samples were determined by the microBCA assay as previously described.

The revised effective cutoff diameter (ECD) of each stage of the impactor at the flow rate of Q, employed in the test was calculated using the formula[39]:

$$D_{50,1} = D_{50,\mathrm{ref}} \left(\frac{Q_{\mathrm{ref}}}{Q_1} \right)^{1/2} \quad (5)$$

where the stage cutoff diameter $D_{50,1}$ at a flow rate Q_1 is related to the stage cutoff diameter ($D_{50,\mathrm{ref}}$) at a reference flow rate (Q_{ref}), using the ECD at 28.3 L/min as a reference. Obtained cutoff values are listed in Table 2.

Several parameters were employed to characterize the resultant deposition profiles of dry powder formulations. These were: the recovered dose (RD), which is the sum of the weights of drug (μg) recovered from inhaler device with its fitted mouthpiece adaptor, induction port, and all stages of the impactor and the emitted dose (ED), which is the amount of drug delivered from the mouthpiece adaptor, induction port, and cone, stages and plates, as well as the filter following simulated inhalation[40]. The percent emission was calculated as the ratio of the ED to the RD. The cumulative mass of powder less than the stated size of each stage of the impactor was calculated and plotted on a log probability scale, as the percentage of total mass recovered in the impactor against the ECD. The fine particle fraction (FPF) was calculated from that plot as the fraction of powder emitted from the inhaler with an aerodynamic diameter <5 μm[41,42]. The experimental mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were also derived by interpolation from this plot. The experimental MMAD of the particles is defined from this graph as the particle size corresponding to the 50% point of the cumulative distribution and the GSD is determined from the slope of this line.

In vitro deposition studies were performed in triplicate and the results presented are average results of the replicated analyses.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) from at least three separate measurements. The statistical difference was measured by using the one-way analysis of variance (ANOVA) followed by post-hoc Tukey multiple comparison test. All analyses were performed by SPSS for Windows statistical software version 11.0. Significance was established when the P-value was <0.05.

Results and discussion

In this study, the *in vitro* aerodynamic characteristics and the aerosol performances of rhIL-2-containing PLGA microparticles alone and in mixtures prepared with mannitol as a coarse carrier were studied. Of the microparticle formulations prepared in our previous studies[28,29], the formulations with suitable particle size for aerosolization and high encapsulation values (Table 1) are selected and aerodynamic studies are performed on these formulations.

Considering their advantages over other inhalation systems and the stability of rhIL-2, which is a protein drug, the inhaler system of microparticles were prepared as DPI system. The stability of the peptides and proteins is limiting the use of nebulizers significantly. Many biopharmaceutics are not stable in their aqueous solutions and due to thermal and surface effects during nebulization, penetration may occur. Generally, due to their sensitivity to penetration in contact with propellants or large air–liquid interfaces, meter dose inhalers (MDIs) are not preferred for peptides/proteins inhalation, neither. The usage of DPI systems for peptides/proteins, however, provides advantages due to the achievement of long-term stability[36]. As the device for the DPI system, Aerolizer® inhaler was used because it provides the opportunity of the relatively easy and effective administration of different doses[43]. Compared with the multidose reservoir systems, the prepacked systems like Aerolizer® have also the advantage in terms

Table 2. Operating conditions and calculated stage effective cutoff diameters of the Andersen cascade impactor.

Andersen cascade impactor	
Flow rate (L/min)	60
Timer per actuation (s)	5
Volume per actuation (L)	5
Cutoff diameter (μm)	
Stage 0	5.8
Stage 1	4.7
Stage 2	3.3
Stage 3	2.1
Stage 4	1.1
Stage 5	0.7
Stage 6	0.4
Stage 7	0.15
Stage 8	Filter

of the protein stability, because it is protected from the environment until use.

As demonstrated in our previous studies, the microparticles prepared by the modified $w_1/o/w_3$ double emulsion solvent extraction method were spherical with a smooth surface. However, at the F4 coded microparticle formulation containing HP-β-CD in the internal aqueous phase have a more porous structure (Figure 2B). The presence of pores on the surface of microparticles containing HP-β-CD is believed to be due to a higher osmotic pressure within the internal water phase. In the double emulsion solvent extraction method, a higher osmotic pressure within the dispersed water phase can give rise to an influx of water toward this aqueous phase with a subsequent increase of the surface porosity[44].

The particle size and size distribution of microparticles were determined by laser diffraction method. Of the microparticle formulations prepared in our previous studies[28,29], the selected formulations have suitable particle size for aerosolization (1.37–5.68 μm). Furthermore, these formulations have high encapsulation values (84.12–99.22%) (Table 1).

The tapped density is an important physical property of dry powders. The tapped density provides significant

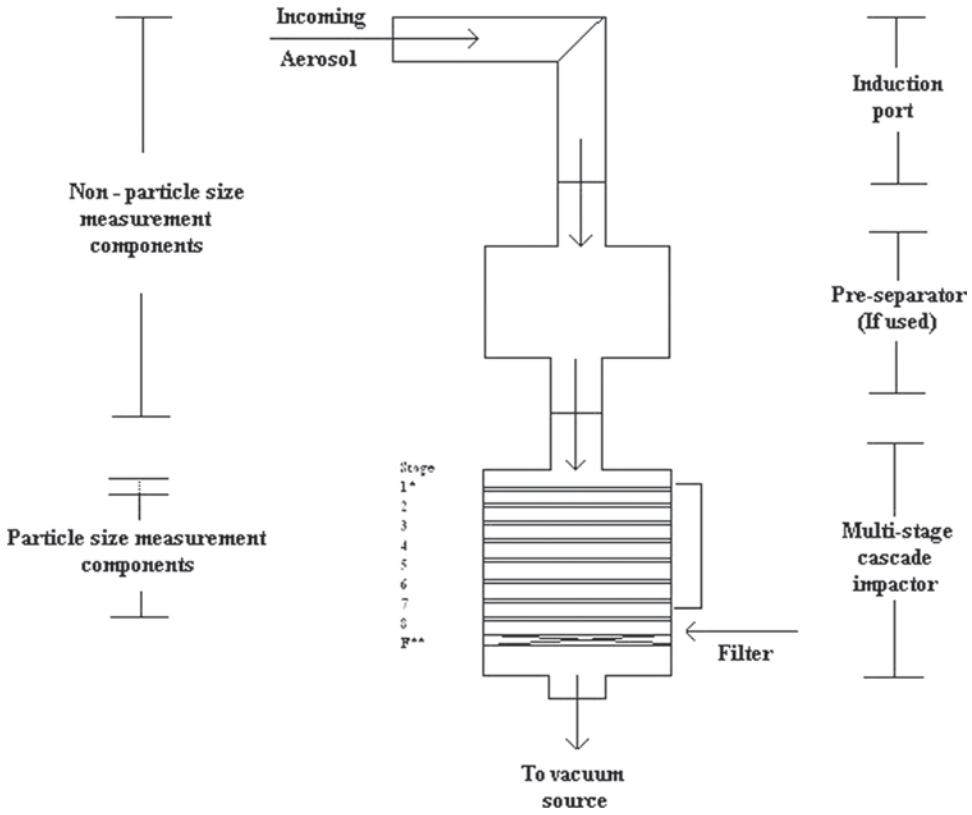

Figure 1. Schematic of the Andersen cascade impactor components.

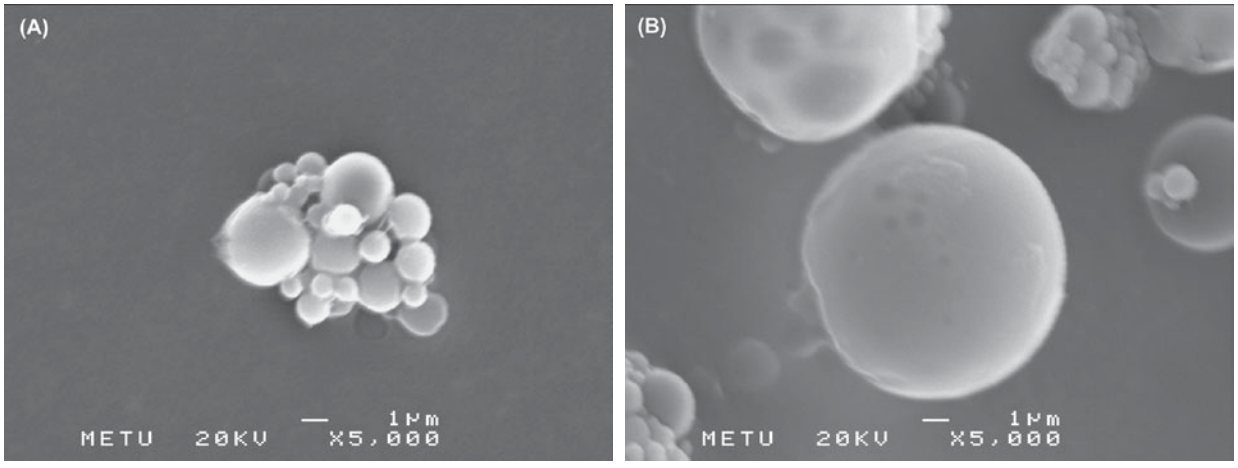

Figure 2. Scanning electron microscopic (SEM) images of rhIL-2-loaded poly(lactic-*co*-glycolic acid) (PLGA) microparticles. (A) F1 and (B) F4.

information about the flow properties of the particles from the inhaler device, the porosity of the particles, the particle size distribution, the true density, and interparticulate cohesive and adhesive forces[37]. A lower tapped density is associated with better aerosolization properties[27,35]. In this study, the tapped density values for the microparticle formulations are found be <0.4 g/cm^3 (Table 3). The lowest tapped density value (0.17 g/cm^3) belongs to the F4 coded formulation containing HP-β-CD. As shown in the SEM photographs (Figure 2B), the HP-β-CD that is added to the internal aqueous phase during the preparation of the microparticles caused the microparticles to have a more porous structure. The increased porosity of the microparticles, however, results in a reduction of the tapped density value. The highest tapped density value (0.30 g/cm^3) was obtained in the F3 coded formulation where a mixture of Resomer RG 752H:Resomer RG 756 in a ratio of 1:1 was used as polymer. It is suggested that using Resomer RG 756, which has a high molecular weight (90 kDa) and a high inherent viscosity (0.77 dL/g), resulted in the formation of a denser matrix structure and subsequently increase in the density of the particles.

Due to the effects of the electrostatic forces of their surfaces, the micronized drug particles generally exhibit cohesive properties. In the dry powder formulations, the coarse carriers that are used together with the micronized drug particles enhance the dose accuracy by improving the flow characteristics of the dry powders[20]. During inhalation, as the micronized drug particles separated from their carrier particles are inhaled, their carrier particles are held in the buccal cavity or oropharynx. Lactose is the most common carrier used in the DPIs. Nevertheless, due to the reductive sugar functions of the carriers like lactose and glucose, it was emphasized that they are not suitable carriers for peptides and proteins[23]. For this reason, in our study mannitol, which in previous studies was determined to be an alternative carrier for peptides and proteins, was used as carrier. Another advantage of the mannitol is that, having a lower humidity sorption capacity than lactose and glucose, it improves the flow characteristics of the powders for inhalation. In the study of Tee et al.[25], as compared with lactose, the utilization of the mannitol enabled the aerosolization performance to increase. In our study, the type of mannitol used was of the average particle size of 50 μm (Pearlitol 50C). Mannitol was geometrically blended with rhIL-2-loaded microparticles to provide a final ratio (mannitol:microparticle) of 9:1 w/w. Content uniformity across each blend indicated a coefficient of variation <5% (Table 3).

Due to the higher density of mannitol (0.67 g/cm^3) compared with the microparticles, the preparation of the physical mixtures of the microparticles with mannitol caused the tapped density values to increase. However, the tapped density values of the dry powder mixtures did not get higher than 0.41 g/cm^3 (Table 4).

Hausner ratio and Carr's index are considered to be indirect methods for quantifying powder flowability. Carr's indexes and Hausner ratios of PLGA microparticles alone and powder mixtures of microparticles with mannitol are listed in Table 4. Carr's index values of the microparticles being higher than 25 indicates that they possess poor flow characteristics. In general, a low bulk and tapped densities indicate poor powder flow[45]. Mixing the microparticles with mannitol allowed a slight reduction of their Carr's index values.

The theoretical primary aerodynamic diameter (MMADt) of each formulation calculated from the geometrical particle diameter and tapped density ranged between 0.72 and 2.78 μm (Figure 3), indicating that the powders were of a suitable size for deposition in the alveolar region of the lung.

In this study, to determine the actual aerodynamic characteristics of the selected microparticle formulations, the eight-stage ACI stated in the EP[38] and USP[45] was used and on the basis of the data obtained in the performed measurements the percent emission, actual MMAD (MMADa) and FPF values were calculated.

Table 3. The percentage uniformity and the coefficient of variation (CV) in rhIL-2 content obtained from the blends containing microparticles and mannitol (values are the mean ± standard deviations, $n = 5$).

Formulation code	% Uniformity	% CV
F1	96.22 ± 1.88	1.95
F2	97.14 ± 1.76	1.81
F3	92.34 ± 2.02	2.19
F4	96.90 ± 1.65	1.70
F5	91.88 ± 2.10	2.28
F6	97.05 ± 1.95	2.01

Table 4. Flow properties of microparticles alone and dry powder formulations containing the blend of microparticles with mannitol (values are the mean ± standard deviations, $n = 3$).

Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Hausner ratio
F1 alone[a]	0.14 ± 0.01	0.20 ± 0.00	30.00 ± 1.00	1.43 ± 0.04
F1 + mannitol[b]	0.24 ± 0.01	0.32 ± 0.01	25.00 ± 0.18	1.33 ± 0.03
F2 alone[a]	0.17 ± 0.00	0.25 ± 0.01	32.00 ± 0.74	1.47 ± 0.08
F2 + mannitol[b]	0.26 ± 0.01	0.36 ± 0.02	27.78 ± 0.44	1.38 ± 0.04
F3 alone[a]	0.21 ± 0.00	0.30 ± 0.01	29.63 ± 1.15	1.42 ± 0.06
F3 + mannitol[b]	0.30 ± 0.00	0.41 ± 0.01	25.64 ± 1.11	1.34 ± 0.06
F4 alone[a]	0.12 ± 0.02	0.17 ± 0.00	29.41 ± 0.71	1.42 ± 0.10
F4 + mannitol[b]	0.22 ± 0.01	0.29 ± 0.00	24.14 ± 0.61	1.32 ± 0.11
F5 alone[a]	0.16 ± 0.01	0.24 ± 0.01	33.33 ± 0.76	1.50 ± 0.08
F5 + mannitol[b]	0.26 ± 0.02	0.35 ± 0.01	25.71 ± 0.28	1.35 ± 0.08
F6 alone[a]	0.18 ± 0.00	0.28 ± 0.00	35.71 ± 0.22	1.56 ± 0.05
F6 + mannitol[b]	0.28 ± 0.00	0.40 ± 0.01	30.00 ± 0.34	1.43 ± 0.06

[a]Microparticles were aerosolized alone without carrier.
[b]DPI formulations containing the blend of microparticles and mannitol.

For all microparticles, the emission values were found to be rather high; during the aerosolization, except for the F3 and F5 coded formulations, in all of the other formulations >90% of the capsule contents released (Table 5). It is suggested that in the F3 coded formulation, due to the high density of the particles, a lower emission value (84.63%) was obtained. In the F5 coded formulation, it is supposed that the chitosan layer on the surface of the microparticles resulted in a reduction of the emission value by increasing the aggregation of the microparticles. The preparation of the mixture of all microparticle formulations with mannitol resulted in a significant increase in the emission value ($P < 0.05$) (Table 5). This increase in the emission value is suggested to arise from the protection of the microparticles by mannitol against aggregation.

In our study, the MMADa values found by measurements performed in the ACI were seen to be higher than the MMADt values (Figure 3). This increase in the MMAD values was suggested to be related to the aggregate formation by the particles during the aerosolization due to the effect of cohesive forces. Sivadas et al.[46] indicated that when the microparticles are aerosolized alone, the forces generated within the DPI are not sufficient to entrain the microparticles and therefore resulted in poor flow characteristics and particle aggregation. The largest MMADa value belongs to the chitosan-coated microparticles. The high MMADa value is believed to be the result of the higher tendency of the microparticles to form aggregates since they are coated with chitosan. The lowest MMADa value (3.95 μm) belongs to the F2 coded formulation.

Mixing the microparticles with mannitol as a coarse carrier allowed the MMADa values to decrease significantly ($P < 0.05$) (Table 5). The mannitol added to the dry powder formulations is supposed to ensure the decrease of the MMADa values by preventing particle aggregation. All MMADa values are found to be lower than 5 μm.

Another important parameter used for the evaluation of the particles' aerodynamic characteristics is the FPF. In our study, the FPF values of the polymeric microparticles were found to be in the range of 52–70% of the total

Table 5. The emission (%), actual mass median aerodynamic diameter (MMADa), geometric standard deviation (GSD), and fine particle fraction (FPF) (%) of microparticles alone and dry powder formulations containing the blend of microparticles with mannitol after aerosolization at a flow rate of 60 L/min (values are the mean ± standard deviations, $n = 3$).

	Parameter			
Formulation code	Emission (%)	MMADa (μm)	GSD (μm)	FPF (%)
F1 alone[a]	91.25 ± 0.65	4.88 ± 0.02	1.19 ± 0.01	58.13 ± 0.07
F1 + mannitol[b]	94.46 ± 0.78	3.49 ± 0.02	1.37 ± 0.01	85.22 ± 0.28
F2 alone[a]	96.75 ± 0.44	3.95 ± 0.03	1.34 ± 0.01	70.57 ± 0.28
F2 + mannitol[b]	98.22 ± 0.23	3.66 ± 0.04	1.23 ± 0.00	80.66 ± 1.01
F3 alone[a]	84.63 ± 1.02	4.87 ± 0.02	1.19 ± 0.00	58.68 ± 0.24
F3 + mannitol[b]	89.23 ± 1.73	4.62 ± 0.04	1.17 ± 0.00	65.50 ± 0.46
F4 alone[a]	90.18 ± 0.98	4.99 ± 0.06	1.20 ± 0.01	58.93 ± 0.76
F4 + mannitol[b]	95.83 ± 1.03	3.89 ± 0.04	1.22 ± 0.01	81.26 ± 0.54
F5 alone[a]	85.44 ± 1.23	5.56 ± 0.03	1.15 ± 0.00	51.95 ± 0.12
F5 + mannitol[b]	90.11 ± 1.43	4.56 ± 0.04	1.20 ± 0.01	63.73 ± 0.62
F6 alone[a]	93.96 ± 0.55	4.33 ± 0.02	1.23 ± 0.01	64.96 ± 0.78
F6 + mannitol[b]	97.34 ± 0.60	3.88 ± 0.06	1.29 ± 0.01	83.05 ± 1.24

[a]Microparticles were aerosolized alone without carrier.
[b]DPI formulations containing the blend of microparticles and mannitol.

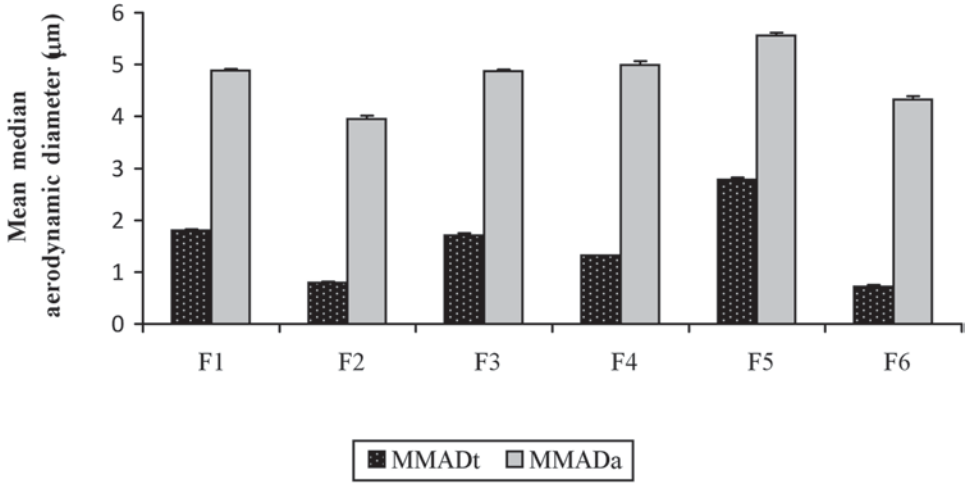

Figure 3. Comparison of the MMADt and MMADa values of rhIL-2-loaded poly(lactic-*co*-glycolic acid) (PLGA) microparticles alone (values are the mean ± standard deviations, $n = 3$).

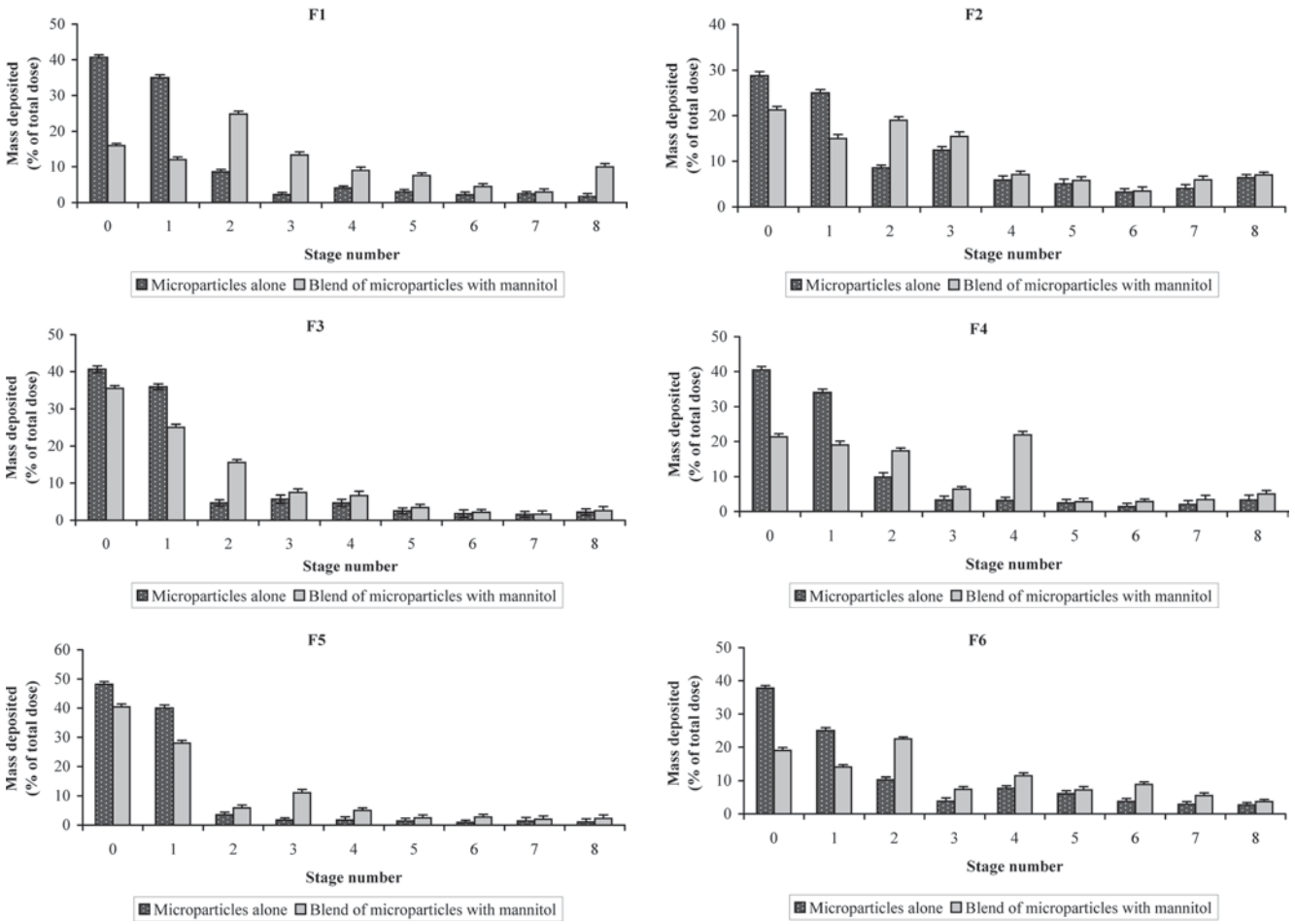

Figure 4. Deposition profile of rhIL-2-loaded poly(lactic-*co*-glycolic acid) (PLGA) microparticles alone and blends containing rhIL-2-loaded PLGA microparticles and mannitol following aerosolization into the Andersen cascade impactor (values are the mean ± standard deviations, $n = 3$). (A) F1, (B) F2, (C) F3, (D) F4, (E) F5, and (F) F6.

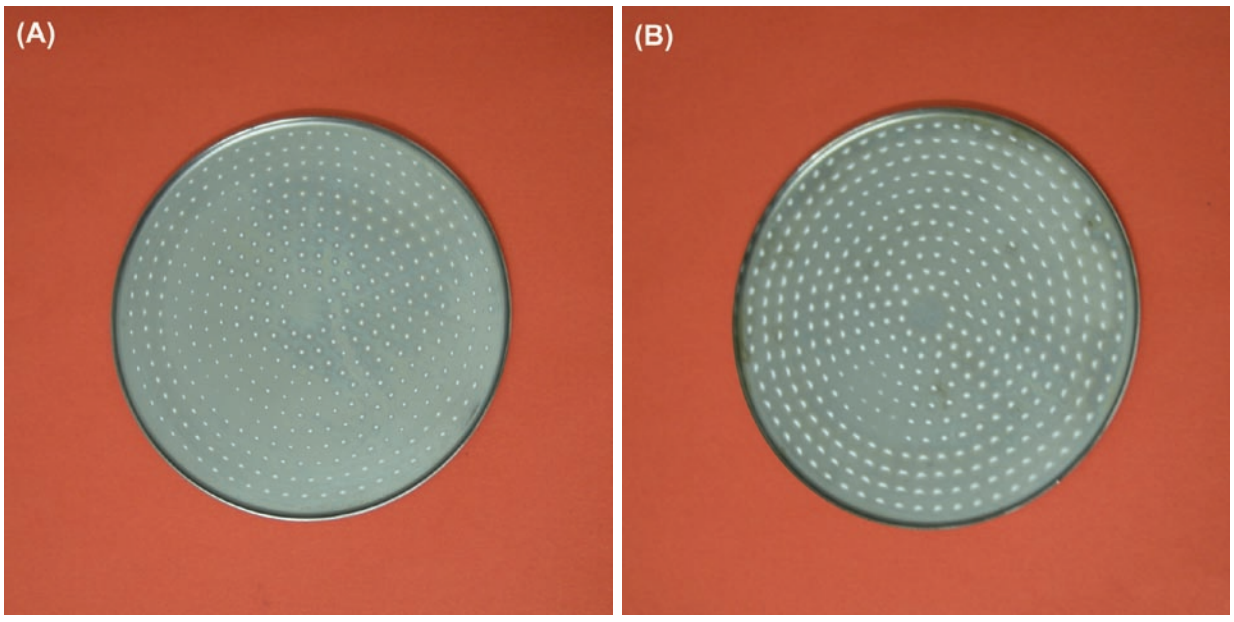

Figure 5 Photographs of stage 7 of the Andersen cascade impactor with (A) F1 microparticle formulation alone and (B) blend of F1 microparticle formulation with mannitol.

loaded dose (Table 5). Similar to the MMADa values, although the highest FPF value (70.57%) is obtained with the F2 coded formulation, the lowest FPF (51.95%) is obtained with the chitosan-coated formulation with the code F5. Otherwise, the FPF values of all microparticles alone are compared with the FPF values of the dry powder formulations prepared by mixing microparticles with mannitol and the increase in the FPF values upon the addition of mannitol is found to be statistically significant ($P < 0.05$) (Table 5). The greatest deposition in the lower stages of the impactor (Figures 4 and 5), and thus the most significant increase in the FPF value is obtained with the F1 coded formulation; mannitol used as coarse carrier caused the FPF to rise up to 85%. This increase in the FPF value is believed to be the result of the prevention of the particle aggregation as a result of mannitol addition in parallel to the decrease in MMAD values. The high FPF value is considered as a result of the optimal aerodynamic characteristics of the microparticles.

In general, the GSD values for the aerosol particles are reported to be in the range of 1.30–3.00[47,48]. In this study, the calculated GSD values for the microparticle formulations are found to be between 1.15 and 1.34. Also, similar GSD values are calculated for the microparticle:mannitol mixtures prepared in a ratio of 1:9 w/w (Table 5).

Conclusion

The MMADt values of each microparticle formulation calculated from the geometrical particle diameter and tapped density indicated that the microparticles were of a suitable size for deposition in the alveolar region of the lung. However, the MMADa values found by measurements performed in the ACI were seen to be higher than the MMADt values. The blending of the microparticles with mannitol as a coarse carrier allowed their MMADa values to decrease and their FPF values to increase. The obtained results have shown that the mixing of microparticles with mannitol possess suitable aerodynamic characteristics for the administration to the lungs by inhalation.

Acknowledgement

The authors wish to thank the Novartis (Turkey) for providing rhIL-2 (Proleukin).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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