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Boosting the aerodynamic properties of vibrating-mesh nebulized polymeric nanosuspensions

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ABSTRACT

Pulmonary application of drug-loaded polymeric nanosuspensions is achieved by vibrating-mesh nebulizers, which allow for an output of intact nanocarriers from the nebulizer reservoir. However, adequate aerosol droplet sizes are a prerequisite for an efficient pulmonary deposition. The current study discloses experimental findings useful to optimize the aerodynamic characteristics of formulations atomized by the vibrating-mesh nebulizers Aeroneb® Pro and eFlow®rapid.

Parameters with significant influence on the aerosol droplet diameter were identified by a statistical design analysis rating size results from laser diffraction. Subsequently, the effect of selected biocompatible solutes on the aerodynamic performance of nebulized formulations was studied and correlated with their physicochemical properties.

Vibrating-mesh generated aerosols were significantly affected by the dynamic viscosity and conductivity of the applied formulation. Consequently, an increase in viscosity enhancer (sucrose and poly(ethylene glycol)) or electrolyte (NaCl and $CaCl_2$) content caused the droplet diameter to decrease. Similarly, purified nanosuspensions revealed a considerable decline in aerosol particle size upon excipient addition. However, coating of polymeric nanoparticles with poloxamer and poly(vinyl alcohol) was necessary to avoid electrolyte-induced nanoparticle aggregation.

Overall, the current study emphasizes that supplementation of nanosuspensions with biocompatible solutes is an excellent means to tailor the characteristics of aerosols generated by vibrating-mesh technology.

1. Introduction

Pulmonary drug application by means of inhalation is known to be a powerful method for the treatment of local and systemic disorders (Olschewski et al., 2002; Patton and Byron, 2007). However, the rapid decay of drug concentration at the target site necessitates several daily inhalations (Gessler et al., 2011). Consequently, numerous controlled drug release formulations have been developed for lung targeting, with biodegradable nanoparticles (NP) most frequently utilized to improve pulmonary therapy (Azarmi et al., 2008; Beck-Broichsitter et al., 2010a, 2012a; Kurmi et al., 2010; Lebhardt et al., 2010; Roa et al., 2011; Rytting et al., 2008, 2010; Ungaro et al., 2012).

Inhalative delivery of NP to the respiratory tract may be accomplished by aerosolization of NP-containing microparticles (composite particles) (Beck-Broichsitter et al., 2012b; Lebhardt et al., 2011) or nebulization of aqueous nanosuspensions (Beck-Broichsitter et al., 2009, 2012c; Dailey et al., 2003a,b; Hureaux et al., 2009). Although the output of intact NP from the nebulizer reservoir represents a key requirement for the application of nebulizer systems (Beck-Broichsitter et al., 2013; Dailey et al., 2003a), the effectiveness of inhalation therapy also depends on the aerodynamic performance of the aerosolized formulation (Beck-Broichsitter et al., 2012a). In contrast to air-jet and ultrasonic nebulizers (Beck-Broichsitter et al., 2013; Dailey et al., 2003a,b; Hureaux et al., 2009), NP formulations were not adversely affected utilizing actively vibrating-mesh technology (Beck-Broichsitter et al., 2009, 2012c, 2013; Hureaux et al., 2009). Moreover, manipulation of the aerodynamic properties of nebulized nanosuspensions seems to be technically feasible by an appropriate selection of dispersion media with tailored physicochemical properties (i.e. viscosity, surface tension, and conductivity) when actuated perforated plates are employed for aerosol generation (Baumann et al., 2012;

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Beck-Broichsitter et al., 2012c; Ghazanfari et al., 2007; Zhang et al., 2007).

Having the impact of aerosol characteristics for pulmonary therapy in mind, a systematic adjustment of aerodynamic performance of nebulized formulations leads to an optimized pulmonary deposition pattern (Carvalho et al., 2011; Hofmann, 2011), and hence, an advanced pulmonary therapy (Groneberg et al., 2003). However, so far only scant information is available describing the effect of excipients on the nebulization performance of aqueous nanosuspensions under application of vibrating-mesh technology (Baumann et al., 2012; Beck-Broichsitter et al., 2012c).

In this regard, the current study aimed to identify the impact of additives (viscosity enhancers, surfactants, and electrolytes) on the aerodynamic characteristics of polymeric nanosuspensions nebulized by the Aeroneb® Pro and eFlow®rapid device. Effects of excipients on aerodynamic characteristics of formulations were investigated by laser diffraction. A statistical design analysis was employed to determine significant parameters influencing the aerosol droplet diameter. Subsequently, a number of biocompatible solutes were tested and results from laser diffraction were correlated with the determined physicochemical properties of samples employed for nebulization. Poly(lactide-*co*-glycolide) NP were coated by hydrophilic polymers (i.e. poloxamer and poly(vinyl alcohol)) to prevent NP aggregation upon electrolyte addition. Finally, the hypothesis was challenged that excipient-supplemented polymeric nanosuspensions reveal improved performance upon vibrating-mesh nebulization.

2. Materials and methods

2.1. Materials

Sucrose, polysorbate 20 (TWEEN® 20), calcium chloride ($CaCl_2$), poly(ethylene glycol) (M_w = 10 kDa) (PEG10 kDa), poloxamer 407 (Pluronic® F127), and poly(vinyl alcohol) (Mowiol® 4-88) were obtained from Sigma-Aldrich (Steinheim, Germany). Sodium chloride (NaCl) was purchased from Carl Roth (Karlsruhe, Germany). Poly(lactide-*co*-glycolide) (PLGA, Resomer® RG502H) was acquired from Boehringer Ingelheim (Ingelheim, Germany). Filtrated, double-distilled water and isotonic saline solution (NaCl 0.9% (m/v)) were procured from B. Braun (Melsungen, Germany). All other chemicals and solvents used in this study were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Physicochemical characterization of fluids prepared for nebulization

Fluids for nebulization studies were prepared with filtrated, double-distilled water. Samples were characterized for density, viscosity, surface tension and conductivity. A detailed description of each method can be found in the Supplementary data.

2.2.2. Nebulization experiments

Nebulization experiments were carried out using two actively vibrating-mesh (i.e. Aeroneb® Pro (Aerogen, Dangan, Galway, Ireland) and eFlow®rapid (PARI, Starnberg, Germany)) devices under the following ambient conditions: temperature: 25 ± 1 °C; relative humidity: 60 ± 10%. NaCl 0.9% (m/v) solution served as a control.

2.2.2.1. Aerosol particle size determination by laser diffraction. The volume median diameter (VMD) of aerosol droplets was determined by laser diffraction (HELOS, Sympatec, Clausthal-Zellerfeld, Germany) (Jaafar-Maalej et al., 2009; Mitchell et al., 2006). The geometric standard deviation (GSD) was calculated from the laser diffraction values according to the following equation

$$\mathrm{GSD} = \sqrt{\frac{d_{84\%}}{d_{16\%}}} \quad (1)$$

where d_n is the diameter at the percentile n of the cumulative distribution.

Table 1
Investigated experimental factors and levels of the central composite design.

	Factor	Level					Unit
		−α	−1	0	1	α	
A	Sucrose	101.7	146.1	219.2	292.3	336.7	mM
B	TWEEN® 20	0.5	1.5	4.5	7.5	8.5	μM
C	NaCl	0.02	0.17	0.42	0.67	0.82	mM
D	Reservoir volume	2.4	3.0	4.0	5.0	5.6	ml

2.2.3. Response surface design

A response surface design in the form of an orthogonal circumscribed central composite design, which includes additional centre and star points (2^4 + 2·4 star points + 4 centre points, α = 1.607), was chosen to study the effect of four factors (i.e. dynamic viscosity (viscosity enhancer: sucrose), surface tension (surfactant: TWEEN® 20), conductivity (electrolyte: NaCl), and reservoir volume) on the VMD of aerosol droplets generated by vibrating-mesh nebulization (Tables 1 and S1) (Box et al., 2005). Intervals were chosen to maintain the orthogonalized values of the design. The order of the experiments was randomized. Statistical calculations were carried out using the software StatGraphics (Statpoint Technologies, Warrenton, USA).

2.2.4. Preparation of NP

NP were prepared by a nanoprecipitation technique (Beck-Broichsitter et al., 2010b, 2013). Briefly, PLGA was dissolved in dimethyl sulfoxide (30 mg/ml) and the resulting polymer solution (1.5 ml) was subsequently injected (injection needle: Fine-Ject® 0.6 × 30 mm) into a magnetically stirred (500 rpm) aqueous phase of 5 ml of filtrated, double-distilled water using a peristaltic pump (flow rate: 10.0 ml/min). The colloidal suspension was dialyzed (Spectra/POR® 6, MWCO: 50 kDa, Breda, Netherlands) against filtrated, double-distilled water to remove the organic solvent. The actual NP concentration in suspension was assessed gravimetrically (BP 211 D, Sartorius, Göttingen, Germany) after lyophilization (ALPHA 1-4 LSC, Christ, Osterode, Germany). NP were characterized and used directly after preparation.

2.2.5. Coating of NP with Pluronic® F127 and Mowiol® 4-88

Freshly prepared nanosuspension was incubated with aqueous Pluronic® F127 and Mowiol® 4-88 solutions (final concentration: 0.1% (m/v)) for 12 h at 25 °C. After incubation, NP were purified from residual polymer by repeated centrifugation (Centrifuge 5418, Eppendorf, Hamburg, Germany) and redispersion cycles at 4 °C and then filtered (5.0 μm, Cameo 30N syringe filters, GE Water & Process Technologies, Ratingen, Germany). The final NP concentration was adjusted by dilution prior to use. Coated NP formulations were characterized and used directly after preparation.

2.2.6. Characterization of NP

2.2.6.1. Size and ζ-potential measurements. The hydrodynamic diameter (d_h) and size distribution (polydispersity index (PDI)) of NP was measured by dynamic light scattering (DLS), and their ζ-potential was determined by laser Doppler anemometry (LDA) in 1 mM NaCl (Zetasizer NanoZS/ZEN3600, Malvern Instruments, Herrenberg, Germany).