European Journal of Pharmaceutics and Biopharmaceutics 119 (2017) 11–16

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Electrolyte type and nozzle composition affect the process of vibrating-membrane nebulization

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ARTICLE INFO

Article history:
Received 8 April 2017
Revised 5 May 2017
Accepted in revised form 8 May 2017
Available online 9 May 2017

Keywords:
Active pharmaceutical ingredients
Aerosol droplets
Electrolytes
Inhalation
Lung delivery
Nozzle coating
Vibrating-membrane nebulization

ABSTRACT

The size of airborne particles determines their deposition pattern within the lungs and therefore, the efficacy of inhalation therapy. The present study analyzed factors affecting liquid atomization performed by vibrating-membrane technology.

First, the process of vibrating-membrane nebulization (eFlow®rapid and Aeroneb® Pro) was challenged with numerous inorganic salts and active pharmaceutical ingredients. All investigated samples caused a sigmoidal decrease in aerosol droplet size upon an increase in concentration. Calculated dose-effect curve characteristics (i.e., half maximal effective sample concentration inducing a halfway drop of the droplet size) indicated distinct molar "potency" amongst the utilized samples with respect to generation of "adequate" inhalation aerosols. Second, the employed solvent (aqueous vs. organic) was shown to amplify the electrolyte effect on vibrating-membrane technology (i.e., dose-effect curve characteristics and overall aerosol droplet size). Third, besides the sample and solvent type, the nozzle composition (diverse metal and polymer coatings) induced a strong impact on the current mode of nebulization. Here, coating materials were identified, which necessitated higher and lower electrolyte concentrations in order to decrease the aerosol droplet size in comparable manner to plain nebulizer membranes. Thus, depending on the employed sample type and concentration, solvent and nozzle composition, a delivery of "inadequate" or "adequate" aerosols for inhalation purpose was observed.

Overall, the current observations could be used to compile suggestions for the rational design of aerosol formulations and nebulizer devices meeting the specific requirements for successful inhalation therapy.

1. Introduction

Vibrating-membrane nebulizers utilize actuated aperture plates, which draw liquid placed in the device reservoir through the membrane to break it up into numerous small droplets [1,2]. Previous studies have affirmed the surprising, but tremendous impact of electrolytes on relevant vibrating-membrane nebulization [3]. Typically, low μM concentrations resulted in a poor nebulizer performance (delivery of large aerosol droplets) [4–9]. The properties of generated aerosol clouds could be tailored to almost any desired value, when spiking the formulations (in)directly with electrolytes [10,11]. It is well understood, that atomization of formulations into micron-scale droplets (i.e., size of ~5 μm) is necessary to allow for enhanced aerosol deposition in the deep lungs following inhalation and hence, an optimized therapy [12,13]. Accordingly, one should determine the specific interaction of the applied sample/medication (i.e., electrolyte type and solvent nature) with the general process of vibrating-membrane nebulization (e.g., quality of the membrane material). Furthermore, one should keep in mind that numerous active pharmaceutical ingredients (APIs) currently utilized for the treatment of lung diseases exist in salt form [7] and thus, might interact with the mode of nebulization leading to "inadequate" (large) or "adequate" (small) aerosol droplets with respect to inhalation therapy.

In this regard, the current study aimed at analyzing the impact of (1) a homologous series of electrolytes and APIs, (2) the employed solvent (aqueous vs. organic) and (3) the nozzle composition (diverse metal and polymer coatings) on vibrating-membrane nebulization in a systematic manner. Delivered aerosol clouds were sized by laser diffraction. Subsequently, the obtained results were correlated with the properties of samples utilized for nebulization.

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http://dx.doi.org/10.1016/j.ejpb.2017.05.004
0939-6411/

2. Materials and methods

2.1. General statement

Some of the employed electrolytes, APIs and solvents represent model systems, which are of no or only of limited eligibility for lung application via the inhalation route of administration.

2.2. Materials

Potassium chloride (KCl), calcium chloride ($CaCl_2$), sodium chloride (NaCl), sodium sulfate (Na_2SO_4), magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$), lithium chloride (LiCl) and sodium bromide (NaBr) were obtained from Carl Roth (Karlsruhe, Germany), while iron(III) chloride ($FeCl_3$) and sodium iodide (NaI) were purchased from KMF Laborchemie (Sankt Augustin, Germany) and Sigma-Aldrich (Steinheim, Germany), respectively. Salbutamol sulfate was a gift from Boehringer Ingelheim (Ingelheim, Germany). Sodium cromoglycate and ipratropium bromide were from Fagron (Barsbüttel, Germany). Sildenafil citrate was from AK Scientific (Union City, USA). Distilled water and N,N-dimethylformamide (DMF) were acquired from B. Braun (Melsungen, Germany) and AppliChem (Darmstadt, Germany), respectively. All chemicals, APIs and solvents were of analytical grade and used as received. The vibrating-membrane nebulizers eFlow®-rapid and Aeroneb® Pro were obtained from PARI (Starnberg, Germany) and Aerogen (Galway, Ireland), respectively.

2.3. Sample preparation and characterization

Salt and API solutions were prepared with distilled water and DMF as solvents. Prior to nebulization all solutions were filtered (1.2 μm; Cameo 30 N syringe filters, GE Waters & Process Technologies, Ratingen, Germany). The current work specifies the salt and API concentration in mM. The salt concentration is further presented as mval/l ($n_{val} = n_{mol} \cdot z$, with n as amount of substance and z as stoichiometric valency). Conductivity measurements were performed using a FiveEasy® conductimeter equipped with a type LE703 conductivity cell (Mettler-Toledo, Giessen, Germany) at 22 ± 1 °C.

2.4. Coating of nebulizer membranes

Firstly, eFlow®rapid membranes were sputter-coated with a layer (~20 nm) of copper (Cu), aluminum (Al) and gold (Au) with an edwards auto 306 (Edwards, Kirchheim, Germany) (Fig. 1).

Secondly, eFlow®rapid membranes were coated with a poly(*p*-xylylene) film [14,15]. Briefly, the nebulizer membranes were wet-chemically conditioned with 3-(trimethoxysilyl)propyl methacrylate to improve the adhesion between the metal surface and the applied polymer. The samples were then coated by vapor phase pyrolysis of di-*p*-xylylene and subsequent chemical vapor deposition according to the Gorham process. The poly(*p*-xylylene) film thickness, which was analyzed using a surface profilometer (Dektak^{3}ST; Veeco Instruments, Mannheim, Germany), amounted to ~500 nm.

2.5. Nebulization experiments

All nebulization experiments were performed under the following ambient conditions: temperature: 22 ± 1 °C; relative humidity: 60 ± 10%.

2.6. Aerosol droplet size

The median geometric diameter (d_g, based on the volume distribution) of the nebulized aerosol droplets was determined by laser diffraction (HELOS, Sympatec, Clausthal-Zellerfeld, Germany) [6–9,14,16]. Briefly, the measurements were performed with six runs of 100 × 50 ms duration during each individual nebulization experiment (lens system: R2, 0.25/0.45–87.5 μm). The diffraction patterns were analyzed in Mie mode, employing the samples' real and complex part of the refractive index. The geometric standard deviation of aerosol clouds (= droplet size distribution) amounted to <2.0 for all investigated samples.

Dose-effect curve characteristics of electrolytes and APIs on the generated aerosol droplet size (i.e., half maximal effective electrolyte and API concentration (EC_{50} value), which induced a halfway drop of the determined aerosol droplet size from an upper to a minimal plateau) were calculated using a sigmoidal dose-response function (Origin 7.0, OriginLab, Northampton, USA).

2.7. Statistical calculations

All measurements were carried out in quadruplicate and values are presented as the mean ± standard deviation (SD). To identify statistically significant differences, one-way ANOVA with Bonferroni's post *t*-test analysis was performed (SigmaStat 3.5, STATCON, Witzenhausen, Germany). A probability value of $p < 0.05$ was considered significant.

3. Results and discussion

Electrolytes have shown significant potential to influence the performance of vibrating-membrane technology, which could be used to produce aerosol clouds suitable for a deposition in the deep lungs [3]. To enhance our knowledge on the applicability and potential limitations of vibrating-membrane nebulization for this purpose, distinct vibrating-membrane nebulizers were challenged with various electrolyte solutions.

In a first set of experiments, the impact of a homologous series of electrolytes on the process of vibrating-membrane nebulization (i.e., eFlow®rapid) was evaluated (Figs. 2–4, Tables 1–3). For all employed salt types a sigmoidal decrease in aerosol droplet size was noticed upon an increase in sample content. Here, the upper plateau region (droplet diameter of ~7–9 μm) observed for "low" electrolyte concentrations was followed by a second plateau (droplet diameter of ~4–5 μm) for "higher" concentrations.

As it can be seen from Fig. 2A, aqueous KCl, $CaCl_2$ and $FeCl_3$ solutions revealed distinct "potency" with respect to decreasing the aerosol droplet size as a function of the applied molar concentration (rank order: $FeCl_3 > CaCl_2 > KCl$). When recalculating the salt content based on the stoichiometric valency (mval/l) the three curves overlapped (Fig. 2B). Table 1 underlined these findings by giving relevant dose-effect curve characteristics for the investigated samples. The EC_{50} values (mM scale) for $CaCl_2$ and $FeCl_3$ were significantly smaller than those observed for aqueous solutions of KCl. However, when analyzing the EC_{50} concentration based on the stoichiometric valency (mval/l scale) more comparable values were obtained.

The effect of the employed salt type on the performance of the eFlow®rapid device was further investigated for a 1.1- (NaCl), 1.2- (Na_2SO_4), 2.1- ($MgCl_2$) and 2.2- ($MgSO_4$) electrolyte (Fig. 3, Table 2). Here, the 1.1-electrolyte NaCl needed the highest concentrations to decrease the generated droplet size (Fig. 3A). Na_2SO_4, $MgCl_2$ and Mg_2SO_4 showed comparable behavior. Again, dose-effect curves overlapped when the stoichiometric valency data were plotted (Fig. 3B). When analyzing the dose-effect curve characteristics for