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The influence of mannitol on morphology and disintegration of spray-dried nano-embedded microparticles

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ABSTRACT

Nano-embedded microparticles represent a promising approach to deliver nanoparticles to the lungs. Microparticles with an appropriate aerodynamic diameter enable an application by dry powder inhaler and the transport of nanoparticles into the airways. By disintegration after deposition, nanoparticles can be released to exhibit their advantages such as a sustained drug release and delivery of the drug across the mucus barrier. The use of an appropriate matrix excipient to embed the nanoparticles is essential for the necessary disintegration and release of nanoparticles.

In this context we investigated the influence of mannitol on the morphology, aerodynamic properties and disintegration behavior of nano-embedded microparticles.

PLGA nanoparticles and mannitol were spray dried each as sole component and in combination in three different ratios. An influence of the mannitol content on the morphology was observed. Pure mannitol microparticles were solid and spherical, while the addition of nanoparticles resulted in raisin-shaped hollow particles. The different morphologies can be explained by diffusion processes of the compounds described by the Péclet-number. All powders showed suitable aerodynamic properties. By dispersion of the powders in simulated lung fluid, initial nanoparticle sizes could be recovered for samples containing mannitol. The fraction of redispersed nanoparticles was increased with increasing mannitol content. To evaluate the disintegration under conditions with higher comparability to the *in vivo* situation, spray-dried powders were exposed to > 90% relative humidity. The disintegration behavior was monitored by analyzing roughness values by white light interferometry and supporting SEM imaging. The exposure to high relative humidity was shown to be sufficient for disintegration of the microparticles containing mannitol, releasing morphologically unchanged nanoparticles. With increasing mannitol content, the disintegration occurred faster and to a higher degree. Under these conditions, microparticles only composed of nanoparticles did not disintegrate.

By enabling the release of nanoparticles from nano-embedded microparticles, mannitol was shown to be an ideal excipient to convert nanoparticles by spray drying into an inhalable dry power formulation.

1. Introduction

In recent years, the preparation of nano-embedded microparticles for pulmonary application gained increasing interest. The combination of particles of two size ranges in one drug delivery system allows to combine the advantages of nano- and microparticles (Ungaro et al., 2012).

Nanoparticles are beneficial for overcoming biological barriers. Pulmonary mucus and bacterial biofilms are strong barriers for drugs applied by inhalation, especially in diseased states such as cystic fibrosis. Drugs suffer from low diffusion rates and a possible deactivation inside mucus and biofilm (Bhat et al., 1996; Forier et al., 2014; Levy, 1986). Nanoparticles have been shown to protect the encapsulated drug from deactivation and in addition are able to permeate mucus and biofilms depending on their physicochemical properties such as size, charge and hydrophobicity (Cone, 2009; Forier et al., 2014; Fröhlich and Roblegg, 2014). Further advantages of nanoparticulate carriers are reduced clearance from the lungs and controlled and sustained drug release (Hadinoto and Cheow, 2014). A sustained release allows the reduction of the dosing frequency and the improved drug transport in mucus and biofilms decreases the required overall dose.

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Although nanoparticles bear the potential of improved pulmonary therapy, their delivery to the lungs is challenging. Particles between 0.1 and 1 µm show low deposition efficiency upon inhalation (Henning et al., 2010; Heyder, 2004). Particles below 100 nm can be deposited to a high degree due to diffusion, while particles larger than 1 µm undergo impaction and sedimentation (Carvalho et al., 2011; Henning et al., 2010; Heyder, 2004; Newman et al., 2009). The application by nebulization of the nanosuspension is possible; however, it also implicates several drawbacks. The development of a long term stable nanosuspension without tendency to microbiological contamination and nanoparticle aggregation or modification is challenging. Furthermore, most nebulizers display disadvantages such as a time-consuming application, high bacterial contamination risk, poor reproducibility and limited portability (Heijerman et al., 2009; Newhouse et al., 2003).

A dry powder formulation based on nanocarriers can be produced by embedding nanoparticles into microparticles. These nano-embedded microparticles (NEM), also called Trojan particles, can be prepared by spray drying of a nanosuspension together with a matrix forming excipient (Bohr et al., 2014; Tsapis et al., 2002; Ungaro et al., 2012). Spray-dried NEM have been prepared with nanoparticles consisting for instance from poly(lactic-*co*-glycolic acid) (PLGA), chitosan, gelatin, poly-ε-caprolactone, polyacrylate, polybutylcyanoacrylate and silica (Grenha et al., 2005; Hadinoto et al., 2007; Kho et al., 2010; Kho and Hadinoto, 2010; Sham et al., 2004; Tomoda et al., 2008).

The choice of an appropriate matrix excipient for the spray drying is crucial for the desired disintegration of the microparticles and release of the nanoparticles in the lungs. Due to their high aqueous solubility and low toxicity, sugars and sugar alcohols such as lactose, trehalose and mannitol are widely used (Grenha et al., 2005; Kho et al., 2010; Kho and Hadinoto, 2010; Sham et al., 2004; Tomoda et al., 2008). Mannitol is especially advantageous for a treatment of bacterial infections in cystic fibrosis and is already approved in the EU as dry powder formulation for the add-on therapy in adult cystic fibrosis patients (Bronchitol®). The osmotic effect is expected to change the viscoelastic properties of mucus and to increase liquid content in mucus and thus the mucociliary clearance (Burness and Keating, 2012). Furthermore, mannitol has been shown to increase the antibiotic sensitivity of *P. aeruginosa* persister bacteria in biofilms (Barraud et al., 2013).

The matrix excipient needs to form bridges between the nanoparticles during spray drying to prevent irreversible agglomeration during the drying process. In contact with liquid, the matrix is supposed to dissolve and to release the nanoparticles. This step is crucial to reach the benefits provided by the nanocarriers such as enhanced penetration of pulmonary biological barriers.

However, the disintegration of microparticles and redispersion of nanoparticles lacks thorough investigation. Most groups investigated the redispersibility behavior by dispersion of the powder in aqueous solution and subsequent determination of size; in some cases the percentage of the redispersed powder was determined as well (Grenha et al., 2005; Kho et al., 2010; Kho and Hadinoto, 2010; Sham et al., 2004; Tomoda et al., 2008). However, the amount of liquid in the lungs is limited. The thickness of the lung lining fluid is around 5–10 µm in the airways and decreasing in the smaller airways and alveoli (Patton, 1996). The total fluid volume in the lungs is estimated to range from 15 to 70 ml (Patton, 1996). Analyzing the redispersibility behavior by dispersing powder in a large volume and applying forces by mixing or stirring might thus not reflect the *in vivo* situation. Ruge et al. addressed this issue by studying the disintegration behavior of NEM after deposition on a mucus layer. Particles, which were easily redispersible in aqueous solution, did not disintegrate on a static mucus layer, while agitation of the mucus enabled redispersion (Ruge et al., 2016). While this model is reflecting the *in vivo* situation, the realization of this experiment may be impeded by the low availability of mucus and the limited comparability of mucus models with biological mucus, respectively. Furthermore, the homogeneous distribution of particles onto the mucus layer requires a complex setup.

In this context we propose a simplified *in vitro* system evaluating the disintegration behavior of nano-embedded microparticles under lung-relevant conditions. Furthermore, we investigated the influence of the mannitol content on morphology, aerodynamic properties and redispersion behavior of nano-embedded microparticles.

2. Materials and methods

2.1. Materials

PLGA (Resomer RG 503H) was purchased from Evonik (Darmstadt, Germany). Coumarin 6 (98%), polyvinylalcohol (Mowiol® 4–88, $M_w \sim 31{,}000$), rhodamine B (for fluorescence), D-mannitol (≥ 98%) and agarose (for electrophoresis) were obtained from Sigma Aldrich (Steinheim, Germany). Ethylacetate (analytical reagent grade) was purchased from Fisher Scientific (Loughborough, UK).

2.2. Nanoparticle preparation

PLGA nanoparticles labelled with coumarin 6 as fluorescence marker were prepared by emulsion-diffusion-evaporation technique. 50 mg PLGA were dissolved in 2.5 ml ethylacetate and 7.5 µl coumarin 6 solution in ethylacetate ($1\ mg \cdot ml^{-1}$) were added. 5 ml 2.5% polyvinylalcohol (PVA) solution were given to the polymer solution and the emulsion was immediately homogenized by ultrasound (30%, 30 s) (Sonopuls UW3100 with MS73 sonotrode, Bandelin electronic, Berlin, Germany). The nanoemulsion was added to 25 ml of water and stirred overnight under light protection for evaporation of ethylacetate. On the next day, total volume was adjusted with water to 40 ml and the nanosuspension was purified by centrifugation (20,000 g, 15 min) and redispersion in 40 ml water. After the second centrifugation, the pellet was redispersed in 7.5 ml water. Concentration of the nanosuspension was determined by freeze drying of an aliquot of the formulation (Alpha 2-4 LSC freeze dryer, Christ, Osterode am Harz, Germany) and documentation of the weight change of the Eppendorf tube. The concentration was adjusted to $5\ mg \cdot ml^{-1}$ by addition of water.

2.3. Nanoparticle characterization

Nanoparticle size and zeta potential were measured by photon correlation spectroscopy (PCS) and laser Doppler velocimetry, respectively, after 1:100 dilution with water with a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK). pH was measured in the undiluted nanosuspension using a Seven compact pH meter (Mettler Toledo, Columbus, USA).

2.4. Preparation of nano-embedded microparticles

An aqueous mannitol solution ($5\ mg \cdot ml^{-1}$) containing $5\ \mu g \cdot ml^{-1}$ rhodamine B was prepared. The spraying liquids were prepared by mixing mannitol solution and PLGA nanosuspension in different ratios, resulting in the following nanoparticle percentages: 0%, 20%, 33%, 50% and 100%. The resulting microparticle powders were named according to their nanoparticle content MP 0, MP 20, MP 33, MP 50 and MP 100, respectively. All samples were spray dried in triplicate. 80 ml of spraying liquid were used for each spray drying experiment and filtrated through Acrodisc® 25 mm syringe filter with 1 µm glass fiber membrane (Pall Life Sciences, Port Washington, USA) to avoid blockage of the spray mesh due to aggregates. Spray drying was performed with a Nano Spray Dryer B-90 (Büchi, Flawil, Switzerland) with tall glass construction, a 7.0 µm spray mesh and the spray head in 45° bent position. The used inlet temperature was 80 °C and the gas flow rate $140\ l \cdot min^{-1}$. All spraying liquids containing nanoparticles were cooled during the spraying process by an ice bath to counteract the temperature increase of the spray head induced by the drying gas