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Development of a spray-drying method for the formulation of respirable microparticles containing ofloxacin–palladium complex

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ABSTRACT

The purpose of this study was to produce low-releasing spray-dried polymeric microparticles (MP) useful to target alveolar macrophages in tuberculosis (TB) inhalation therapy.

Ofloxacin (Ofx) was encapsulated as ofloxacin–palladium (Ofx–Pd) complex into poly DL-lactide (PLA) MP by spray-drying. Ofx–Pd was prepared according to a method previously reported. A D-optimal design was employed to optimize drug content (DC), aerodynamic diameter (d_{ae}) and span. d_{ae} was calculated coupling tap-density to particle size analysis. The MP were characterized by SEM, UV spectrophotometry, and DSC. *In vitro* drug release was performed in comparison to Ofx loaded PLA MP.

The Ofx–Pd complex formed spontaneously with a 1:1 stoichiometry. Inlet temperature, drug loading and polymer concentration resulted the most influential. Optimal MP had span of 0.9, a round shape, d_{ae} of 2.5 μm, and DC of 30% (w/w). DSC and SEM analyses correlated with particle size. The optimized MP formulation showed a very low release at pH 7.4 compared to spray-dried Ofx loaded MP, the release increased slightly at lower pHs.

Potentially inhalable MP were obtained by an optimized spray-drying process. The very low initial drug release at physiologic pH could be useful to target alveolar macrophages and to avoid systemic exposure.

1. Introduction

In recent years, particular attention has been focused on the lungs as a new site for drug administration (Patton and Byron, 2007). Much research work has been directed to the development of new drug delivery systems for the treatment of pulmonary lung infections to achieve local drug accumulation and to reduce systemic exposure (Lu and Hickey, 2007; Hickey, 1996). This approach can be worthwhile especially for TB treatment, even though, so far, many pitfalls have hindered the success of the anti-TB inhalation approach. Several solutions to this problem have been proposed. These include the use of PLGA microparticles (MP) (O'Hara and Hickey, 2000; Garcia-Contreras et al., 2006; Hirota et al., 2007), large porous particles (Giovagnoli et al., 2007), liposomes (Giovagnoli et al., 2003; Orozco et al., 1986; Gilbert, 1996; Vyas et al., 2005) and polymeric nanoparticles (Pandey et al., 2003; Sharma et al., 2004).

The reason of such efforts resides in the role of alveolar macrophages as primary site of TB infection. Due to its peculiar membrane, the Mycobacterium tuberculosis can survive within cells inhibiting lysosome fusion and this pathogen peculiarity makes alveolar macrophages therapeutic targets (Leemans et al., 2005). The conventional TB therapy is troublesome and rather ineffective as far as intracellular delivery is being concerned (Hartkoorna et al., 2007; Blasi et al., 2009). Inhalation may enhance drug accumulation in the lungs overcoming some of the well known drawbacks of TB therapy, such as high dose and toxicity, and allowing drug access to macrophages. Of course, to do so is required the drug to avoid rapid absorption into the blood stream. This goal can be achieved by using microencapsulation approaches that, however, cannot always prevent rapid drug release (Allison, 2008). This can be especially true when dealing with inhalable powders, which are mainly prepared by spray-drying (Ye et al., 2010). This process, although fast and potentially scalable, is not hundred percent able to ensure confinement of drugs within the polymeric matrix. As a result, drugs can be sometimes found on the surface of the particles provoking a high burst release. As an adjunct to microencapsulation, modification of drug solubility may be useful to control burst release, especially when dealing with actives, such as ofloxacin (Ofx) (Macìas et al., 2001). Ofx is a second generation fluoroquinolone used in the second-line therapy of multi-drug-resistant TB (MDR-TB). Ofx, being sparingly soluble in water, does not fit the requirement for optimal intracellular delivery.

In order to meet this objective, a new approach was attempted by developing low-releasing MP able to target alveolar macrophages.

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The strategy is based upon the formation of an insoluble Ofx complex with palladium (Pd) (Vieira et al., 2009a) to be entrapped in PLA MP by spray-drying. A number of studies reported on the complexation of fluoroquinolones with metal cations as a means to modulate solubility and antibacterial activity (Vieira et al., 2009b). In the case of Ofx, Pd salts have been found to produce insoluble precipitates upon reaction of the drug suspension with the metal salt solution (Sagdinc and Bayari, 2004).

Pd has been used to prepare drug–metal complexes with antibacterial, antifungal, antitumor activity (Garoufis et al., 2009; Kovala-Demertzi et al., 2001; Farhad et al., 2009; Abu-Surrah et al., 2008). Of course when dealing with such compounds, a major concern is the potential Pd accumulation in the body upon administration of Pd containing salts (Iavicoli et al., 2009, 2010) and, in particular, upon chronic exposure (Iavicoli et al., 2008). Pd toxicity is poorly addressed and inconsistent results are present in the literature, especially as far as inhalation is being concerned (EMEA London, 2007; Gaworski et al., 2008; Yokohra et al., 2008). For this reason, toxicity studies will be the subject of future work to address the use of such complexes in inhalation therapy.

Such complexes can be useful to enhance intracellular penetration of inhaled particles by virtue of the low solubility and thereby high retention within the MP matrix. This may favor alveolar macrophage targeting as a result of activation and increased uptake by the infected cells.

The present work focused on the development of Ofx–Pd loaded MP by encapsulating an Ofx–Pd complex opportunely synthesized in order to achieve a low-releasing preparation. For this purpose, a design-of-experiment (DoE) approach was applied to investigate the main variables influencing the spray-drying preparation process so as to obtain respirable MP potentially useful in TB inhalation therapy.

2. Materials and methods

2.1. Materials

Ofx and K_2PdCl_4 were purchased from Sigma Aldrich (Milan, Italy). Poly DL-lactide (PLA) (MW 29,000 Da, Resomer 203H) was supplied by Boehringer, Ingelheim (Ingelheim, Germany). Hydrochloric acid and sodium hydroxide were obtained from Carlo Erba (Milan, Italy). Acetonitrile and acetone were purchased from J.T. Baker (Milan, Italy). All water was Milli-Q grade (Milli-Q System, Millipore, Milan, Italy). When not specified, all other chemicals and reagents were of the highest purity grade commercially available.

2.2. Synthesis of Ofx–Pd complex

The Ofx–Pd complex was prepared by dropping an Ofx water dispersion into a K_2PdCl_4 solution under mixing (Vieira et al., 2009a). Appropriate amount of Ofx was suspended in water, which was then added to the aqueous solution of K_2PdCl_4 at room temperature. Immediately after the addition of the Ofx suspension, a precipitate formed. The reaction mixture was maintained under stirring at room temperature for 60 min. The suspension obtained was centrifuged for 10 min at 4000 rpm and 20 °C using a Universal 32R Hettich zentrifugen (Tuttlingen, Germany). The solid obtained was washed with water, centrifuged and dried for 15 h under vacuum by Edwards high vacuum pump EDM2.

2.3. Preparation of Ofx–Pd complex microparticles

Ofx–Pd was encapsulated into PLA MP by spray-drying of an Ofx–Pd dispersion in acetonitrile polymer solution (total volume 13.5 mL). Spray-drying was performed by using a Mini Spray dryer model B-290 (Büchi, Italy). A 0.7 mm nozzle tip and a 1.5 mm diameter nozzle screw cap were used. The aspirator capacity was set to 20 m^3/h. The remaining operating parameters were changed according to the design of experiment presented below. The Ofx–Pd MP were separated from the drying gas by a high-performance cyclone (Büchi, Italy), since previous studies have indicated an improved efficiency of this cyclone compared to a regular cyclone in collecting particles less than 2 μm in diameter (Brandenberger, 2003).

Similarly, Ofx loaded MP were prepared using the following spray-drying conditions established by previous studies: inlet temperature 90 °C, air flow rate 536 L/h, feed rate 2.4 mL/min, aspirator 28 m^3/h.

2.4. D-optimal design

The Design Expert software (version 8.0.1) was used to build up the DoE approach to model the Ofx–Pd MP spray-drying preparation process. A D-optimal design was employed for process optimization and three instrumental and two formulation parameters were chosen as independent variables: inlet temperature (A), liquid feed rate (B), air flow rate (C), drug loading (D) and polymer concentration (E) (Table 1). A general quadratic polynomial model was chosen to fit experimental data (Eq. (1)).

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} \sum_{j=1}^{k} b_{ij} x_i x_j + \sum_{i=1}^{k} b_{ii} x_i^2 + e \qquad (1)$$

where y is the response, b_0, b_i, b_{ii} and b_{ij} are the intercept, linear, quadratic and interaction regression coefficients correlated to the x_i, x_j level of the ith, jth factor as reported in Table 1 and e is the residual random error of the model.

High and low levels of the parameters above mentioned were chosen according to previous observations and the physicochemical properties of the materials employed. In this regard, the inlet temperature upper limit was selected on the basis of the acetonitrile boiling temperature (81.6 °C), while the liquid feed rate of 3.2 mL/min was the highest value affording the production of dry powders without formation of droplets on the wall of the drying chamber when the outlet temperature was at the lowest limit. The formulations were prepared and spray-dried in randomized manner to eliminate bias. The design table is reported in Table 1.

The measured and calculated dependent variables were the aerodynamic size expressed as d_{ae} of Ofx–Pd MP, size distribution dispersity, referred as span, drug content (DC) expressed as % (w/w) of Ofx–Pd per mg of dry powder (Eq. (2)). Three replicates for each measurement were performed.

$$\%\text{Drug content} = \left(\frac{\text{mg Ofx–Pd}}{\text{mg dry powder}}\right) \cdot 100 \qquad (2)$$

The models were statistically evaluated by ANOVA and response surfaces were built to draw the 3D response profile against the variables employed. The surfaces obtained for the three responses were combined by the overall desirability approach (Derringer and Suich, 1980), which is summarized by Eq. (3):

$$f(d_{(g)}) = \frac{\sum_{i=1}^{M} w_i d_i}{\sum_{i=1}^{M} w_i} \qquad (3)$$

Table 1
Independent variables of the D-optimal design.

	Factors	Low limit (−1)	High limit (1)	Units
A	Inlet temperature	75	90	°C
B	Feed rate	1.6	3.2	mL/min
C	Air flow rate	246	473	L/h
D	Drug loading	5	20	% (w/w)
E	Polymer concentration	1	4	% (w/v)