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Development of PLGA dry powder microparticles by supercritical CO_2-assisted spray-drying for potential vaccine delivery to the lungs

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ABSTRACT

In this work, biocompatible and biodegradable poly(D-L-lactide-co-glycolide) (PLGA) composite microparticles with potential use as carrier for vaccines and other drugs to the lungs were developed using supercritical CO_2-assisted spray-drying (SASD). Bovine serum albumin (BSA) was chosen as model vaccine, and L-leucine as a dispersibility enhancer, and their effects on the particle characteristics were evaluated. The dry powder formulations (DPFs) were characterized in terms of their morphology and aerodynamic performance using an *in vitro* aerosolization study – Andersen cascade impactor (ACI) − to obtain data such as the fine particle fraction (FPF) with percentages up to 43.4%, and the mass median aerodynamic diameter (MMAD) values between the 1.7 and 3.5 μm. Additionally, pharmacokinetic and cytotoxicity studies were performed confirming that the produced particles have all the necessary requirements for potential pulmonary delivery.

1. Introduction

The local application of drugs to the respiratory tract via inhalation facilitates a site specific treatment of lung diseases with higher treatment efficacy, lower systemic exposure and consequently, reduced side effects [1,2]. It is also an advantageous route for systemic drug delivery since it shows a high solute permeability which facilitates gas exchange via diffusion [3], due to its very thin absorption membrane (0.1–0.2 μm), to its elevated blood flow (5L/min) and to the highly vascularized alveolar epithelium constituted by a single layer of cells, which offers a large absorptive surface (80–100 m^2) [4–6]. It also has an easy administration, shows early effects of drugs' pharmacological actions and has no risk of drug decomposition [7,8]. Furthermore, unlike the oral route, it is not subject to first pass metabolism which is especially important to macromolecules (i.e. peptides and proteins) that are easily degraded by enzymes [9]. Controlled drug release systems composed of polymeric materials with particular characteristics, such as biocompatibility and degradability, have been shown to improve the pharmacokinetic and pharmacodynamic profiles of encapsulated drugs in the lung [1,7,10].

Vaccines are responsible for death prevention associated with numerous infection diseases every year, even though there is still a huge amount of children morbidity due to vaccine-preventable diseases [11]. Vaccines administered parenterally require expensive cold chain transport and trained personnel, and they can induce needle-stick injuries with possible transmission of viruses [12,13]. To treat the infectious diseases that affect poor populations the vaccines should be simple, cheap, easy to produce and stable. This is all possible with pulmonary vaccination added to all the advantages for drug delivery already mentioned [14]. Above that is the fact that the lungs have gained a lot of attention given that the respiratory tract is the main entry of pathogens and also it has an extensive dendritic cell network lining the airway epithelium that facilitates a first line of defence for antigens [15].

To efficiently deliver powder formulation to the lungs, an inhaler should generate an aerosol of a suitable size with a reproducible drug dosing while ensuring chemical stability and activity [5]. The dry powder inhaler (DPI), presents all these characteristics, being also propellant-free [16,17]. As for the delivery of vaccines this device is very suitable once macromolecules (polysaccharides, proteins and peptides) tend to degrade when in a liquid solution and so are provided with a greater stability [12].

In order to understand where particles with different size deposit in the respiratory tract, the aerodynamic diameter (d_a) has to be taken into account and is defined by the equation

$$d_a = d_g \sqrt{\frac{\rho_p}{\rho_o \chi}}$$

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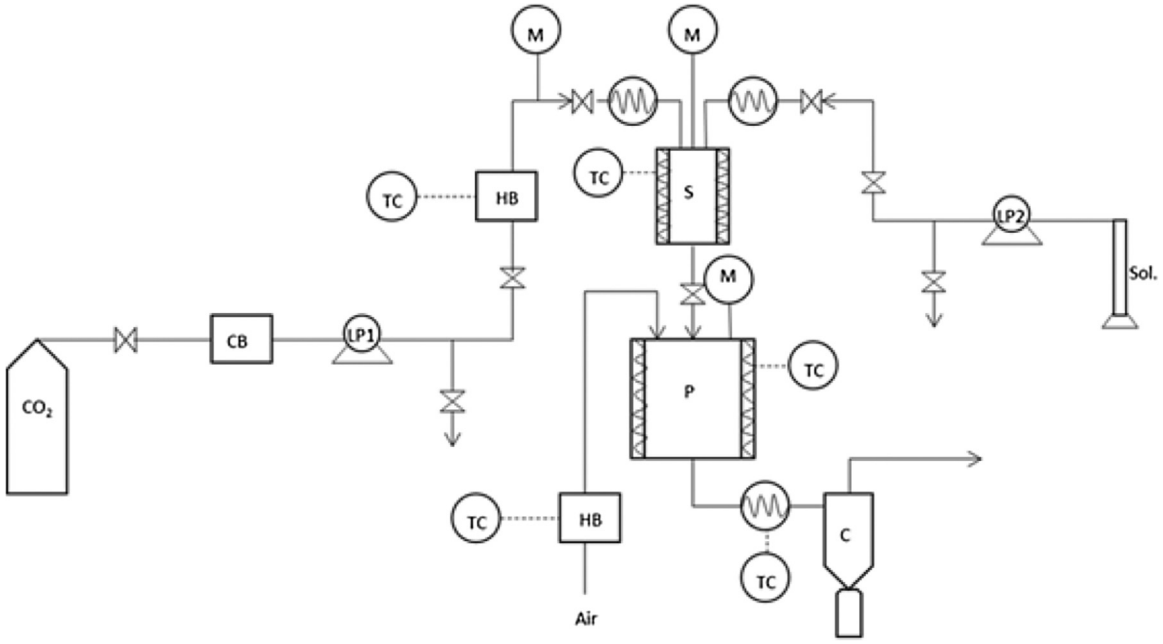

Fig. 1. Schematic representation of the SASD apparatus: (CB) cryogenic bath; (LP) liquid pump; (HB) heating bath; (TC) temperature controller; (M) manometer; (S) saturator; (P) precipitator; (c) cyclone.

Where d_g is the geometric diameter, ρ is the mass density of the particle, ρ_o is the unit density and χ the particle dynamic shape factor [4].

Though some conflicts regarding the range for optimal d_a to efficiently reach the deep lungs may exist, the most recent works state that the aerodynamic diameter of aerosol particles should be between 1 and 5 μm since this size range is the one that reaches to the deep lungs [18–20].

Poly(lactic-co-glycolic acid) (PLGA) is used as a biodegradable slow-release polymer, it is physically strong, with low toxicity, highly biocompatible and bioabsorbable and also allows an effective encapsulation of all kinds of drugs, proteins and various other macromolecules such as DNA, RNA and peptides [21–23]. Additionally, it was approved by the FDA (Food and Drug Administration) for drug targeting in the year 2000 [23], being one of the first among all the new minimally invasive protein delivery system options, which makes it one of the most promising materials in medical and biotechnological research with high commercial interest [24,25].

More recently, alternative particle production methods using supercritical fluids (SCF), especially supercritical carbon dioxide ($scCO_2$), have been proposed to overcome some limitations. Conventional methods usually require large amounts of organic solvents and thus involve additional extensive purification steps to remove the residual solvent. Carbon dioxide is the most used SCF, since it is nontoxic, non-flammable, inexpensive and readily available in high purity from a variety of sources [26–28]. Besides, it has a low critical point (31.1 °C and 73.8 bar), which is suitable to use with heat-sensitive materials, being able to reduce manufacturing complexity and energy [29]. Supercritical assisted atomization (SAA) or supercritical assisted spray-drying (SASD) is based on the solubility of controlled quantities of $scCO_2$ in a liquid solution containing the drug and excipient. The mixing happens in a heated saturator with a large contacting surface, assuring a high residence time and a near-equilibrium solubilisation of the CO_2 [30,31].

In this work, composite particles using PLGA as an excipient were produced using SASD. Bovine serum albumin (BSA) was used as a model antigen [32], and L-leucine as a dispersibility enhancer [33] in order to develop pulmonary vaccines. Furthermore, all the particles were properly characterized to make sure they were suitable for pulmonary delivery.

2. Experimental section

2.1. Materials

Poly(D-L-lactide-co-glycolide) (PLGA, PURASORB® PDLG 5002A, molar ratio: 50/50, inherent viscosity 0.21 dL/g) was purchased from PURAC (Gorinchem, Netherlands). Bovine Serum Albumin (BSA, Mw 60 kDa), L-Leucine (> 98%) and acetonitrile were obtained from Sigma-Aldrich. Industrial carbon dioxide (purity ≥ 99.93%) was obtained from Air Liquide.

2.2. Design of experiment

A design of experiment (DoE) approach was adopted using Statistica™ software version 10 (Statsoft, Bell, Tulsa, Oklahoma) to understand the effect of some of the formulation variables on the particles size [34,35]. In this case the absence or presence of both L-leucine and BSA as well as two different CO_2-to-liquid solution volumetric flow ratios (8.3 and 5). All the experiments were made in triplicate and the statistical differences in between different formulations was obtained using ANOVA. The analysis was performed considering changes in $D_{v,50}$, MMAD and FPF.

2.3. Microparticles preparation

A solution of 0.47% (w/v) PLGA, 0.09% (w/v) BSA and 0.02% (w/v) L-leucine was prepared with 83% (v/v) acetonitrile and 16% (v/v) distilled water. While the PLGA was dissolved in part of the acetonitrile, both the BSA and leucine were dissolved in water. After full dissolution, the aqueous solution was added to the PLGA solution under stirring, with the rest of the acetonitrile added in the end.

Particles were produced using a laboratory scale SASD apparatus, represented in Fig. 1 [36–38]. Two high-pressure pumps to deliver the liquid solution (HPLC pump 305 Gilson) and the CO_2 (HPLC pump K-501, Knauer) to the static mixer. Since the CO_2 pump is suitable for liquid solutions, there is a need to liquefy the CO_2 in a cryogenic bath. Before entering the static mixer, the CO_2 is heated in an oil bath. The static mixer (3/16 model 37-03-075 Chemieer) is a high-pressure vessel with a 4.8 mm diameter, 191 mm length and 27 helical mixing