J. of Supercritical Fluids 95 (2014) 106–117

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

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

Preparation of rifampicin/poly(D,L-lactice) nanoparticles for sustained release by supercritical assisted atomization technique

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ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 5 August 2014
Accepted 8 August 2014
Available online 18 August 2014

Keywords:
Rifampicin
Poly(D,L-lactide)
Supercritical assisted atomization (SAA)
Nano-encapsulation

ABSTRACT

In this work supercritical assisted atomization (SAA) process was used for the co-precipitation of poly(D,L-lactide) (PDLLA) and rifampicin (RIF) as nanoparticles for sustained release applications. The effect of the variation of PDLLA/RIF ratio on co-precipitate characteristics was mainly investigated. The precipitated particles were analyzed in terms of their morphological, thermodynamic and crystallographic properties. In addition, loading efficiency and in-vitro release studies were conducted. Spherical PDLLA/RIF nanoparticles with mean diameter ranging from 123 to 148 nm were prepared. Loading efficiency was greater than 100% resulting in RIF loadings of 28.8 to 50.5%. X-ray diffraction revealed that the encapsulated RIF is in an amorphous state, while NMR spectra indicated no structural modifications after the SAA process. In-vitro release studies showed an initial burst release of 80–87% of total RIF loaded, necessary to suppress the generation of resistance by the microorganism, followed by first-order sustained release between 0.4 and 0.8 mg/L RIF per day over a period of 17 days.

1. Introduction

Tuberculosis (TB) is the second largest cause of death in the world from a single infectious agent. The World Health Organization (WHO) estimates that, due to its infectious nature, approximately one third of the world population is latently infected by its causative agent, *Mycobacterium tuberculosis* and approximately 9 million new cases are reported each year [1].

Rifampicin (RIF) is one of the most powerful antibiotics against bacterial pathogens [2] since it can diffuse easily into tissue, living cells and bacteria, making it highly effective against pathogens such as *M. tuberculosis*. The bactericidal activity of RIF is due to its binding to the bacterial DNA-dependant RNA polymerase and its inhibition [3]. However, despite being an effective drug for TB, there are some challenges in treating TB with RIF. First, its bactericidal activity is directly proportional to its concentration at the target site [4]. Due to its poor water solubility, dissolution in biological liquids is low and can limit its ability to reach the required concentration. Second, long term continuous therapy leads to the risk of hepatoxicity in many patients [5]. Third, anti-TB therapy is complex and prolonged, usually 6–9 months, with a high pill burden [6,7]. This leads to patient non-compliance and the subsequent emergence of multidrug-resistant TB [8].

The current strategy for limiting adverse side-effects and enhancing therapeutic activity of anti-TB drugs is via encapsulation within a carrier, from where it is released in a controlled way over an extended period of time. This technique results in improved patient compliance, improved bioavailability, lower dose, lower cost and lower toxic side effects [9]. Currently, the most popular techniques for the preparation of encapsulated RIF in polymeric carriers are the double emulsion solvent evaporation and spray drying. These techniques are able to produce particles of RIF encapsulated in biodegradable polymeric carriers, such as: poly(lactide-*co*-glycolide) (PLG) [10,11], poly(lactide) (PLA), poly(*n*-butylcyanoacrylate) (PBCA) [12], poly(isobutylcyanoacrylate) (PIBCA) [12], alginate [13] and gelatine [14] with particle sizes ranging between 0.2 and 5 μm and encapsulation efficiencies between 10 and 70%. However, these processes have some drawbacks: for example, the double-emulsion solvent-evaporation technique is complex and involves multiple steps, requires the use of surfactants and solvent removal usually takes up to 12 h [15–17].

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http://dx.doi.org/10.1016/j.supflu.2014.08.004
0896-8446/

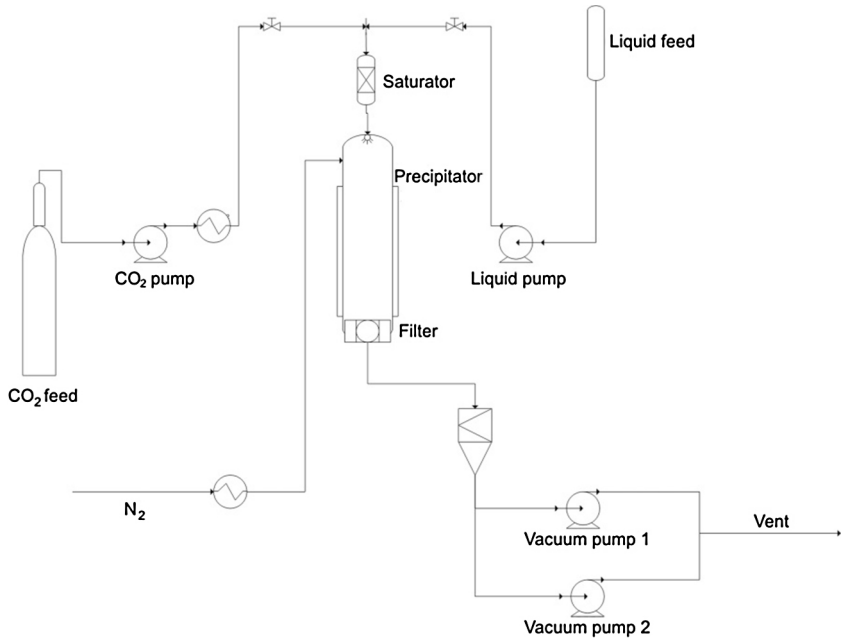

Fig. 1. Schematic representation of the SAA apparatus.

Spray drying often requires high temperatures; it is difficult to obtain spherical particles [18] and a secondary drying step can be required to reduce residual solvent to acceptable levels [19,20].

Alternative processing techniques in which supercritical carbon dioxide (sc-CO_2) plays a key role in the preparation of pharmaceutical products have become well established over the last years [21,22]. The unique properties of supercritical carbon dioxide such as, tunable solvent power, liquid-like density, gas-like diffusivity, together with its non-toxicity and low cost, allowed this technology to be successfully proposed in several pharmaceutical processes such as micronization [23,24], modification [25,26] and encapsulation [26,27] of drugs. Typical sc-CO_2 based processes include rapid expansion of supercritical solutions (RESS) [28], rapid expansion of supercritical solutions into liquid solvents (RESOLV) [29], particles from gas saturated solutions (PGSS) [30] and several anti-solvent techniques such as supercritical anti-solvent (SAS) [31], gas anti-solvent (GAS) [32] and solution-enhanced dispersion by supercritical fluids (SEDS) [33]. While many of these processes have been applied for preparation of controlled release particles, few have been able to produce particles in the nanosize range [29,34].

However, the most attractive micronization technique using sc-CO_2 is the Supercritical Assisted Atomization (SAA). It is based on the formation of an expanded liquid solution formed by an organic solvent, one or more solutes and sc-CO_2. The expanded liquid is, then, atomized in a precipitation vessel. When compared to ordinary atomization techniques, SAA has the advantage of operating on a reduced viscosity and low surface tension medium, due to the specific characteristic of expanded liquids and, thus, produces an enhanced atomization due to reduced cohesive forces. SAA has been successfully applied to the micronization of several active principles [35–38] and has been particularly successful in the production of polymer + drug micrometric co-precipitates [39–41]. It is also possible to operate at reduced pressure to produce particles of thermo-sensitive compounds [42–44]. SAA technique was previously used to produce micrometric RIF particles [24,45] but, at the best of our knowledge, has never been used to produce polymer-RIF co-precipitates.

Therefore, the aim of this work was to evaluate whether the SAA process can be applied for the preparation of RIF-loaded polymer nanoparticles. poly(D,L-lactide) (PDLLA) was used as matrix for RIF. The effect of the variation of polymer/drug ratio on the co-precipitate characteristics was investigated. The precipitated particles were analyzed in terms of morphological, thermodynamic and crystallographic properties. In addition, loading efficiency and in-vitro release studies were conducted.

2. Materials and methods

2.1. Materials

The PDLLA homopolymer (Resomer® R 203 H, M_w: 28k) was supplied by Evonik, Germany. Rifampicin (823 g/mol) was purchased from Sigma-Aldrich (South Africa). Dichloromethane (DCM) and acetone were supplied by Sigma Aldrich (Milan, Italy). CO_2 (purity 99.9%) was purchased from SON (Naples, Italy). All material was used as received.

2.2. SAA apparatus

SAA apparatus (Fig. 1) consists of two high pressure pumps (mod. 305, Gilson) delivering liquid solution and CO_2 to the saturator. The saturator is a high pressure vessel (25 cm^3 internal volume) loaded with stainless steel perforated saddles, which assured a large contact surface between the liquid solution and CO_2. The expanded liquid obtained in the saturator is sprayed through a thin wall injection nozzle (80 μm internal diameter) into the precipitator (3 dm^3 internal volume). A controlled flow of N_2 is taken from a cylinder, heated in an electric heat exchanger (mod. CBEN 24G6, Watlow) and sent to the precipitator to induce droplet evaporation. The saturator and the precipitator are electrically heated using thin band heaters (Watlow, mod. STB3EA10). A stainless steel filter, located at the bottom of the precipitator, allowed powder collection and the gaseous stream flow out. To operate below the atmospheric pressure, the plant is equipped with a vacuum system, formed by two vacuum pumps (DVP mod. ZA100P) operating downstream of the precipitator. The system is completed by a condenser, which separates liquids from the gas stream.