ELABORATION OF ANTI-TUBERCULOUS DRUG DELIVERY SYSTEMS THROUGH CLASSIC AND IMPINGING-JET SAS PROCESSES

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ABTRACT

The current strategy for limiting the adverse side-effects and enhancing therapeutic activity of anti-tuberculous (anti-TB) drugs is *via* encapsulation within a carrier from where it is released in a controlled fashion over an extended period of time. Currently, the most popular techniques for the preparation of encapsulated anti-TB drugs in polymeric carriers are the double emulsion solvent evaporation and spray drying techniques. However, these processes require many processing steps; they expose the drugs to high temperatures and/or require significant post processing time to remove residual solvent. An alternative process is via the supercritical anti-solvent (SAS) method which is a one-step process for particle design. The SAS process is conventionally used either for precipitation of pure compounds or co-precipitation of drugs and excipients. The co-precipitation of a polymer and a drug with the classic SAS process poses a challenge as differences in nucleation starting points and rates could result in separate precipitation of drug and polymer. Classic SAS modified with and impinging jet introduction device (IJ-SAS) has shown before to improve mixing between components and has the potential to improve the drug embedding during the co-precipitation of polymer/drug systems. The aim of this work is to elaborate anti-TB drug delivery systems. For that purpose, the ability to produce co-precipitates of anti-TB drugs, respectively, namely isoniazid (INH) and rifampicin (RIF), with poly(L-lactide) (PLLA) was tested with both classic and IJ-SAS process. The organic solvent used was methylene chloride (DCM). The following parameters were kept constant for both processes: pressure (10 MPa), temperature (308 K), molar ratio DCM:CO_2 (0.05:1) and polymer:drug ratio (4:1). For classic SAS capillary diameter was of 127 μm and the organic solution velocity was of 4 m/s. For IJ-SAS the following process parameters were varied: capillary diameter (254 μm and 127 μm) and organic solution velocity (1 and 4 m/s). Solvents used were dichloromethane for RIF/PLLA and dichloromethane/acetone (9/1 by volume) for INH/PLLA co-precipitation. SEM observations indicated the formation of spherical particles in all instances with particle diameters ranging from 0.8 to 4 μm. DSC thermographs showed that the PLLA remained semi-crystalline, while XRD analysis suggested that the RIF were in the amorphous state. Loading efficiency of RIF into the PLLA matrix has been improved when the IJ-SAS was employed, indicating better mixing.

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 potent antibiotics against bacterial pathogens [2] since it can diffuse easily into tissue, living cells and bacteria, making it highly effective against pathogens such as *Mycobacterium tuberculosis* [3]. The bactericidal activity of RIF is due to its binding to, and inhibition of, the bacterial DNA-dependant RNA polymerase [4]. Isoniazid (INH) is also used as an antimycobacterial agent for the first line therapy of TB. INH possesses a relatively short half-life ranging from 1 to 4h [5], depending on the rate of metabolism and is freely soluble in water. Severe metabolic acidosis, acetonuria, and hyperglycemia have also been reported [6].

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 [7]. Currently, the most popular techniques for the preparation of encapsulated anti-TB drug in polymeric carriers are the double-emulsion solvent evaporation[8] and spray drying techniques[9].

Alternative processing techniques in which supercritical carbon dioxide plays a key role in the preparation of pharmaceutical products have become well established over the last years [10]. 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 various pharmaceutical processes such as micronization [11], modification [12] and encapsulation [13] of drugs. In typical supercritical CO_2–based processes, CO2 can play the role of a solvent in rapid expansion of supercritical solutions processes (RESS) [14], of a dispersion agent in particles from gas saturated solutions processes (PGSS) [15] or as an anti-solvent in supercritical anti-solvent processes (SAS) [16].Several derivatives of these main three categories were developed [17][18]. Among them, impinging jet technique, in which two jets are introduced in face to face position, was applied to SAS processes (IJ-SAS) in order to enhance transfer phenomena and to favour intense mixing conditions [19].

The aim of this work was to elaborate current anti-TB drug delivery systems by co-precipitation of anti-TB drugs, RIF and INH, with a biodegradable polymer, poly(L-lactide) using both classic and impinging jet SAS processes.

# MATERIALS AND METHODS

1. Materials
Poly-L-lactide (Resomer L206S) was supplied by Evonik, Germany. Rifampicin (RIF) and isoniazid (INH) were purchased from Sigma-Aldrich (South Africa). Dichloromethane (DCM) (purity > 99.9 %) and acetone (purity > 99.5 %) were supplied by Sigma Aldrich (France). CO_2 (purity of 99.7%) was purchased from Air Liquide (France). All material was used as received.

2. SAS and impinging jet-SAS set-up

The experimental set-up used for the SAS and IJ-SAS processes is illustrated in **Figure 1**. It is mainly composed of a 1 liter high pressure precipitation vessel (Top Industrie S. A., France) equipped with a double jacket connected to a thermostated bath. This autoclave is fed with CO_2 through a regulated high pressure piston pump (LGP50, Separex, France) and with the organic solution through a HPLC pump (Gilson 307, France). The outlet of the autoclave is equipped with a back pressure regulator (Tescom, Germany) to control the pressure during the experiments.

In a typical SAS experiment, the autoclave is first set to the desired temperature and then fed with preheated CO_2 with a regulated mass flow rate. The pressure inside the autoclave is controlled by adjusting the aperture of the back pressure regulator. When the operating parameters, i.e. pressure, temperature and CO_2 flow rate are reached, the organic solvent was dispersed through a capillary nozzle in a co-current $SC-CO_2$ stream to reach the desired solvent/CO_2 molar ratio in the vessel. The organic solvent is then replaced with the organic solution. Precipitation process is then carried out for a fixed time defined by the final quantity of product to collect. Finally, a washing step was realized by flowing pure CO_2 in the autoclave in order to remove the remaining solvent. Once the washing completed, the autoclave was slowly depressurized, and micronized particles were recovered.

In IJ-SAS process, the experimental protocol is almost the same expect that the organic solution was introduced through the impinging jet capillary and was impinged with CO_2 at the same kinetic energy. Therefore, The CO_2 flow at the inlet of the autoclave was splitted between the capillary in the top of the autoclave and the impinging jet capillary.
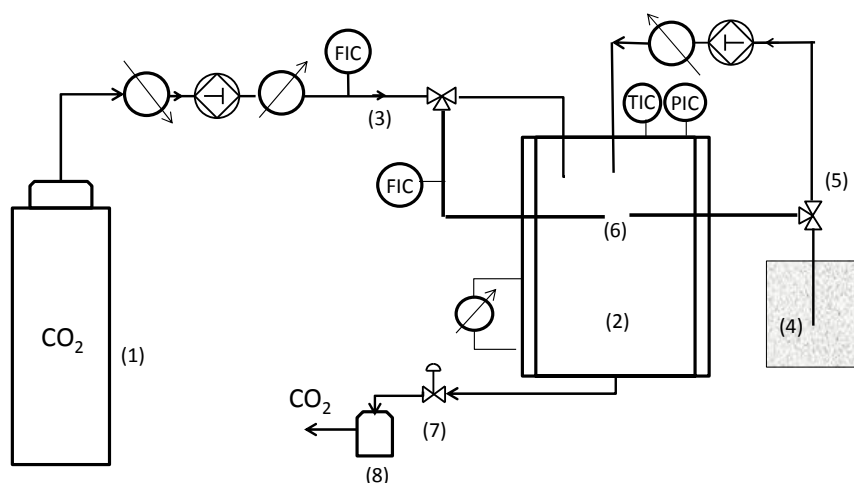
Figure 1: (1) CO_2 bottle; (2) High pressure cell; (3) CO_2 injection line; (4) Organic solution ; (5) Organic solution injection line; (6) Impinging jets capillaries; (7) Backpressure regulator; (8) Solvent trap

3. Analytical Methods

Samples were observed by a JEOL 7500 F field–emission scanning electron microscope (FE–SEM) at an accelerating voltage of 2 kV. The sample was mounted on the Aluminium stub using a carbon tape. The samples were sputter coated by carbon to avoid charging. Particle Size (PS) and Particle Size Distribution (PSD) were estimated from SEM images using the Image Pro Premier software (Media Cybernetics, Bethesda, MD, USA). Approximately 500

particles of each formulation were measured to calculate PSD. Crystallographic analyses of samples were performed on a powder X-ray diffractometer (XRD, (XPERT PRO PANanalytical, Netherlands). The measurement conditions were a Cu K α radiation generated at 45 kV and 40 mA as X-ray source in the range 2–50° (2θ) and step angle 0.0131°/s.

The entrapment of RIF in the particles was determined by weighing a sample of the particles and dissolving in 10 ml of DCM. The absorbance of the solution was measured with a UV/VIS spectrometer (Lamda 35, Perkin Elmer) at a wavelength of 474 nm. The entrapment of INH in the particles was determined by lysing them with 5% SDS (w/w) in 0.1 N NaOH and then adjusting to pH = 7 before UV/VIS analysis at 263 nm. Drug concentration in the solutions was determined by using calibration curves, which were prepared by plotting concentration versus absorbance data. Drug loading (% w/w) was therefore calculated according to Eq. (1).

$$DL\% = \frac{The\ mass\ of\ RIF\ loaded}{The\ total\ mass\ of\ particles}\ x\ 100\%$$ (Eq. 1)

In-vitro release studies: Into 50 ml Nalgene® centrifuge tubes were placed 25 ml of a 0.01 M phosphate buffered saline (pH = 7.4) and 1 mg/ml Tween 80. For RIF/PLLA particles 0.2 mg/ml ascorbic acid was added as an antioxidant. A known mass of particles was suspended in the media after which the tubes were sealed and placed in a shaker bath at 100 RPM and 37°C. At given time intervals, the tubes were centrifuged at 5000 rpm for 15 min and 5 ml of the supernatant removed for analysis. The remainder of the supernatant was removed and the microsphere pellets were re-suspended in fresh PBS (25 ml), similar to the method used by Liggens and Burt [20]. The amount of drug in 5 ml of the supernatant was determined with UV/VIS analysis, according to the standard curves of the respective drugs.

RESULTS

The process settings and solvent used in the encapsulation experiments are summarised in Table 1.

Table 1: Process operating conditions used for the co-precipitation of RIF and INH with PLLA

Reference	Method	Solvent	dc^* (mm)	P (MPa)	T (°C)	w * (%)	m_{PLLA}/m_{RIF}	X* (%)	U (m/s)
RIF/PLLA_1	Classic SAS	DCM	127	10	35	0.5	4	5	4
RIF/PLLA_2	IJ – SAS	DCM	254	10	35	0.5	4	5	1
RIF/PLLA_3	IJ – SAS	DCM	127	10	35	0.5	4	5	4
INH/PLLA_1	Classic SAS	DCM/acetone	127	10	35	0.5	4	5	4
INH/PLLA_2	IJ – SAS	DCM/acetone	254	10	35	0.5	4	5	1
INH/PLLA_3	IJ – SAS	DCM/acetone	127	10	35	0.5	4	5	4

* Dc: capillary diameter; u: velocity of the organic solution; w: mass fraction of polymer in the organic solution; X: solvent/CO_2 molar ratio; u: velocity of the organic solution.

1. Particle Morphology

SEM images of processed RIF/PLLA and INH/PLLA after SAS processing according to the process conditions shown in Table 1 are shown in Figure 2.
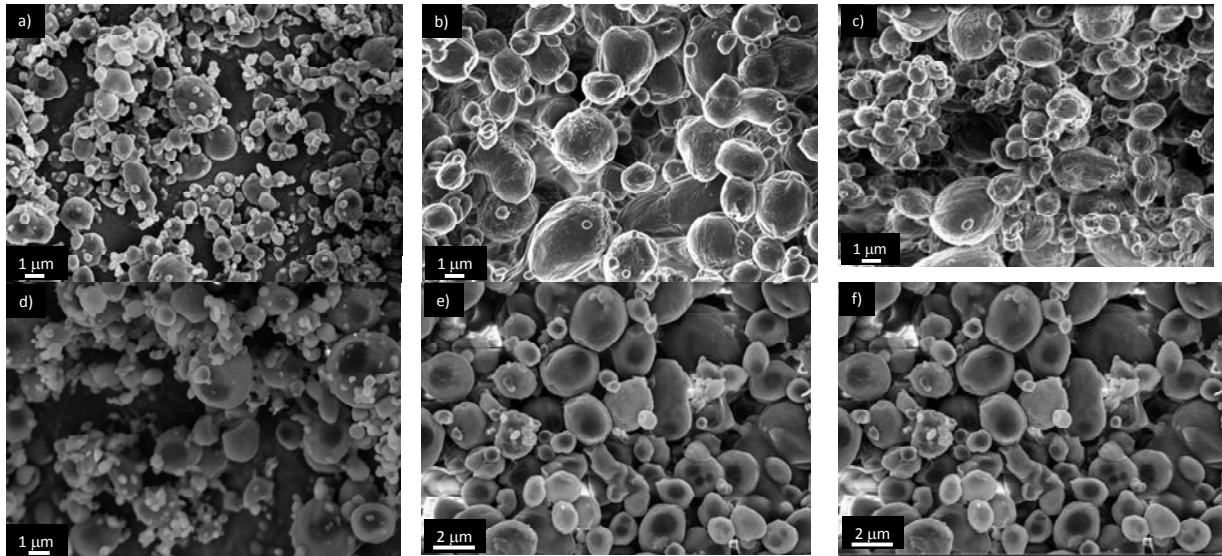
Figure 2: SEM micrographs of RIF/PLLA_1 (a), RIF/PLLA_2 (b), RIF/PLLA_3 (c), INH/PLLA_1 (d), INH/PLLA_2 (e) and INH/PLLA_3 (f).

All particles are discrete and have a spherical shape. No indications of discrete crystalline drug particles are observed. Average diameters and polydispersity indices are presented in Table 2.

Table 2: Mean particle diameter and polydispersity index of particles produced from the RIF/PLLA and INH/PLLA co-precipitation experiments

Formulation	Mean particle diameter (µm)	Polydispersity Index
RIF/PLLA_1	0.79	0.39
RIF/PLLA_2	1.77	0.25
RIF/PLLA_3	1.92	0.42
INH/PLLA_1	0.87	0.44
INH/PLLA_2	1.41	0.42
INH/PLLA_3	1.54	0.39

The RIF/PLLA formulations showed a pale orange colour, suggesting the presence of RIF (which has a characteristic dark red colour), diluted by the presence of the white polymer. Since INH is naturally white, it is not possible to draw the same conclusion from the INH/PLLA formulations.

Contrary to expected results, coprecipitates formed with classic SAS are smaller than that obtained with IJ-SAS. In the same conditions of injection, more uniform particles are obtained in IJ-SAS configuration (lower polydispersity index). The same tendancy was observed for RIF/PLLA and INH/PLLA coprecipitation experiments.

2. Drug Loading

Drug loading in the different drug/PLLA formulations were quantified in order to detect the presence of drug in the obtained particles, separate or uniformly mixed with PLLA. For INH/PLLA coprecipitates, INH was not detected with spectrophotometric analyses. Therefore, only drug loadings (wt% in product) in RIF/PLLA coprecipitates were compared

to the initial drug content (wt% of total drug to polymer in the solution) prior to the SAS process in Table 3.

Table 3: Comparison of drug content in solution versus drug loading in the RIF/PDLLA formulations:

| Formulation	Drug content wt%	Drug loading (wt%)
RIF/PLLA_1	20	0.60
RIF/PLLA_2	20	1.41
RIF/PLLA_3	20	2.75

For the different tested conditions, no trace of INH in INH/PLLA coprecipitates was observed, and drug loading in RIF/PLLA coprecipitates was very low (<3%). An explanation of this result could be the very low supersaturations of each drug in the fluid phase in the tested conditions. Indeed, concentrations of RIF and INH in the organic solutions were very low (respectively 1.67 mg/ml and 1.59 mg/ml). In addition to the high quantity of drug dissolved in the fluid phase, in low supersaturation conditions, very small particles can be formed since the crystal growth does not occur and can be driven out of the autoclave by the fluid phase flow through the frit (0.2 µm cutoff). The very low drug content in RIF/PLLA is in good agreement with those obtained by Patomchaiviwat et al. [38]. Authors found that low concentrations of rifampicin in DCM/PLLA solutions lead to low drug content.

In order to confirm these results, classic SAS micronisation expriments were carried out for each drug in the same experimental conditions than coprecipitation tests. No INH was collected in the autoclave and micronisation yield of RIF was very low (< 5%). These results are therefore coherent with the coprecipitation ones.

In IJ-SAS process, the slight increase in RIF loading could be explained by the enhancement of the mixing conditions which favours the trapping of the precipitated rifampicin in PLLA.

3. X-ray Diffraction

XRD scans of the unprocessed RIF and INH were compared with the RIF/PLLA and INH/PLLA formulations prepared via SAS and IJ-SAS (Figure. 3). The spectra of the unprocessed RIF and INH show the characteristic peaks of these drugs in the crystalline state. None of these peaks are present in any of the RIF/PLLA or INH/PLLA formulations.
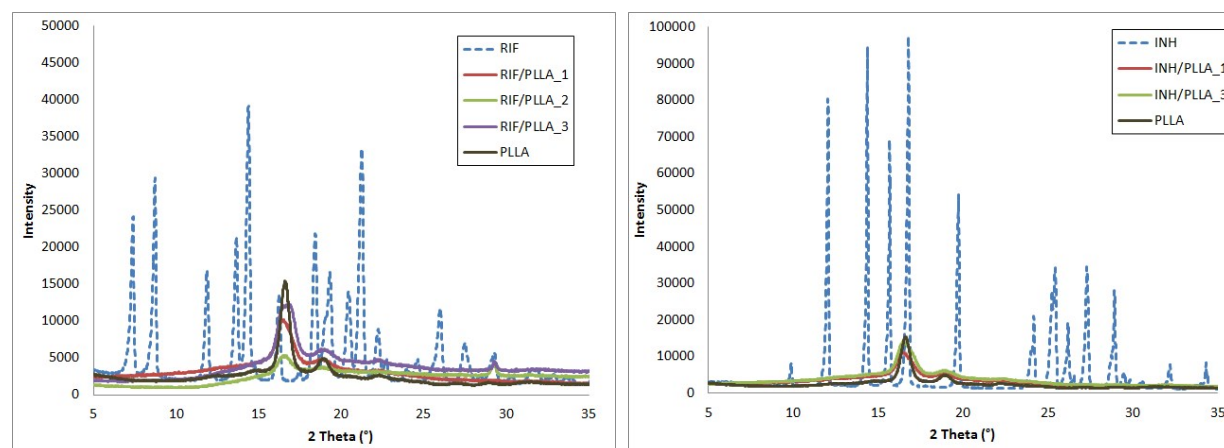
Figure 3: X-ray diffraction patterns of the RIF/PLLA and INH/PLLA formulations.

4. In-vitro Release Studies

Drug release studies were carried out on the different RIF/PLLA co-precipitates prepared with IJ-SAS.
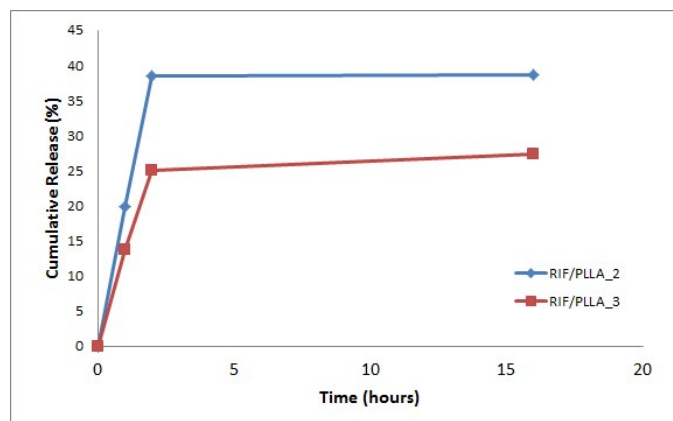
Figure 4: In-vitro release profiles from RIF/PLLA co-precipitates from IJ-SAS.

Both co-precipitate formulations showed a bimodal release pattern with an initial rapid release followed by a sustained release (Figure 4). The higher release of RIF/PLLA_2 could be due to the slightly smaller mean particle diameter presenting as higher surface area to the PBS media. Due to the low initial drug content entrapped in the PLLA particles combined with the slower release after 16 hours it was not possible to measure accurate values using UV-VIS spectroscopy.

CONCLUSION

Two anti-TB drugs, rifampicin and isoniazid, were coprecipitated with poly(L-lactide) in order to elaborate drug delivery system. Both classic and impinging jet SAS processes were studied. For INH/PLLA coprecipitation tests, no INH was detected in the final collected powders, while very small drug loadings were obtained in RIF/PLLA coprecipitates. In the considered experimental conditions, the initial concentrations of drugs in the organic solutions were very low, leading to low supersaturations of the drugs in the fluid phase. IJ-SAS process was shown to slightly increase the drug loading for RIF/PLLA formulations. In the studied conditions, spherical particles were obtained in all instances with particle diameters ranging from 0.8 to 4μm. DSC thermographs showed that the PLLA remained semi-crystalline, while XRD analysis suggested that the RIF was in an amorphous state.

Further studies should be carried out notably by increasing the concentration of the drug in the initial organic solution in order to enhance the drug precipitation and loading in PLLA.

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