Rifampicin nanosuspension production using microfluidic device

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ABSTRACT

The aim of this work is to study the rifampicin recrystallization using microfluidic devices. The microfluidic device was produced in glass substrates and the geometry patterned was designed to enhance hydrodynamic flow focusing phenomena. The results showed that is possible to obtain nanoparticles with low polidispersivity. Besides the small size, the original crystalline structure changes resulting in an amorphous structure. These results could improve the Rifampicin dissolution rate, therefore improving the drug bioavailability. These studies are still in progress and other results will be published in the future.

Key Words: Rifampicin, nanoparticles, microfluidic devices

INTRODUCTION

Rifampicin is commonly used in Tuberculosis treatment and presents variability in its bioavailability [1]. This drug has a complex structure and several polymorphic forms, and is known to have poor bioavailability. The polymorphism contributes to difficult the drug dissolution. [2,3]

The microfluidic devices offer nanoparticles production in one step bottom up technique. This approach render a continuum process with low polidispersivity, low consumption of reagents and additives [4]. Drug nanosuspensions are constituted by particles in nanometric scale with increased surface area which can improve the dissolution rate and drug bioavailability [5,6]. The antisolvent precipitation technique is one of the mechanisms used to obtain nanoparticles and can be improved by using microfluidic devices [7], obtaining particles with a smaller nucleation size [8].

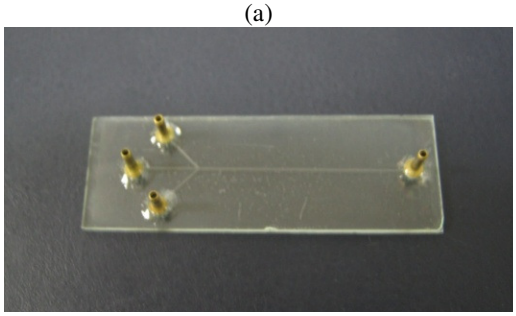
In this work the results of a study of Rifampicin nanosuspension production through antisolvent technique are presented, using microfluidic devices. The particle size and morphology were evaluated by observing the influence of fluid flow rates, the relation between flow rates, the presence of surfactants and Rifampicin concentrations.

EXPERIMENTAL

In this paper, commercially soda lime microscope glass slides (76 x 26 x 1 mm, Knittel, Germany) were used to obtain the microfluidic devices. The glasses were patterned with hydrofluoridric acid solution combined with chloridric acid to improve the etching process (HF:HCl:H_2O - 1:2:3).

To seal the channels with a cover glass, UV glue diluted in 50 % pure acetone was used. Hydrodinamic flow focusing geometry was patterned to produce Rifampicin Nanosuspension by nanoprecipitation phenomena and the device is shown in Figure 1(a) and in (b) the device at work. The center channel has 100 µm of width and the side channels have 110 µm. The device depth was 80µm.

(a)



(b)

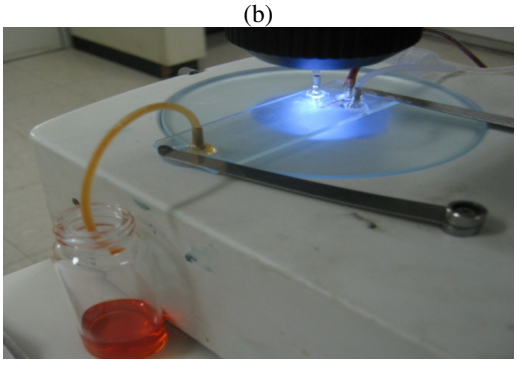


Figure 1. The microfluidic device: (a) geometry design in detail and (b) the device in operation

Methanol was used as a solvent for Rifampicin and was introduced in the center channel as disperse phase (Qd). Water was used as anti-solvent and was introduced as continuous phase (Qc) in side microchannels. The fluids are

introduced by syringe pumps. The Tween 20 was used as surfactant and the influence was evaluated in the final results. In table 1 are shown the complete set of experimental procedure.

Table 1. Experimental Set Conditions

Experiment	A	B	C
Continuous Fluid Flow rate (µL/min)	100	150	200
Fluid Flow ratio (Q_c/Q_d)	10	30	50
Surfactant [Tween20](v/v %)	0	0,5	1
Rifampicin concentration (g/mL)	10	30	50

Besides the particle size measurement through dynamic light scattering technique (DLS, Malvern Instruments), the flow focalization behavior in the microchannel was observed using an optical microscope (Coleman, XTB-2T) with 10 up to 40-fold increase. The Rifampicin nanosuspensions were filtered in a hydrophilic membrane with 0.22 µm of pore (Millipore Ind.). The Rifampicin powder was maintained at membrane for FEG-SEM analysis.

The crystalline structures were examined using wide X-ray diffraction (XRD-6000, Shimadzu) to observe the diffraction profile of rifampicin processed in microfluidic channels. XRD data was collected over an angular range from 5 to 50 º (2θ) in continuous scan mode using a step size of 0.02º.

RESULTS

With the microfluidic devices it was possible to obtain particles sizes in the range of 100-770 nm and the polidispersivity value below 0,3. We can see in figure 2 the Rifampicin hydrodynamic focalization in two conditions, in 2(a) the ratio Qc/Qd was 50 (Experiment C) and in 2(b) was 10 (Experiment A). Using the thinnest disperse phase flow condition (ie. Qc/Qd= 50) is possible to obtain smaller particles. The total fluid flow rate did not interfere in the particle sizes, however the use of experiment C conditions can offer more particles production rates.

The presence of surfactant helped to reduce the rifampicin size, although the amount of Tween 20 in water phase is very low (1%v/v). In addition, the rifampicin concentration did not interfere in the particle size, so the use of more concentrated values, as in Experiment C, offer more particles production in the final suspension.

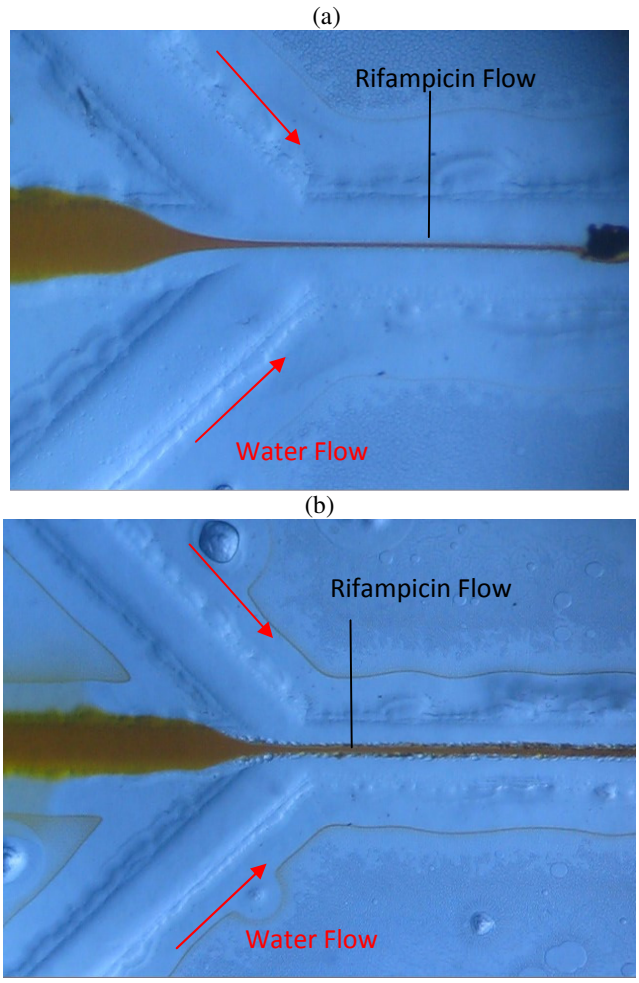


Figure 2. Rifampicin hydrodynamic flow focalization: (a) Qc/Qd =50 (Exp. C) and (b) Qc/Qd =10 (Exp. A).

In Figure 3 is shown the size measurement obtained with DLS technique. In Figure 3 (a) the particle has 500 nm of diameter with 0.2 of polidispersivity obtained when the Qc/Qd was 10 (Experiment A) and in (b) 210 nm with also 0.2 of polidispersivity with a Qc/Qd equal to 50 (Experiment C).

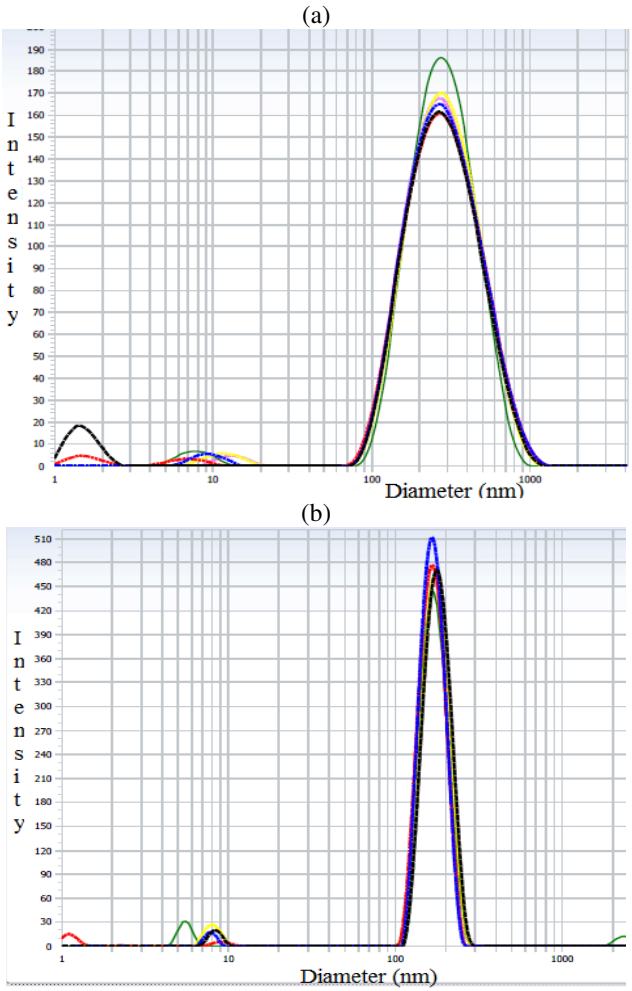


Figure 3. Particle sizes for different conditions

In Figure 4 a micrograph of Rifampicin nanoparticles obtained with a FEG-SEM. In (a) Raw Rifampicin (see Dias. S [1]) is depicted and in (b) and (c) is presented processed Rifampicin in microfluidic devices in the conditions presented before, where the measured size was about 208 nm. It is important to note that the particles obtained in microfluidic process have a spherical shape compared to commercial raw Rifampicin. This shape suggests an amorphous structure of fabricated nanoparticles via microfluidics.

In Figure 5 a XRD profile is presented comparing a raw Rifampicin with a Rifampicin processed in microfluidic device. Raw Rifampicin have the presence of characteristics peaks that indicates a polymorphous crystal. Observing the curve for Rifampicin processed in microfluidic system we can see the absence of peaks, so we have an amorphous particle.

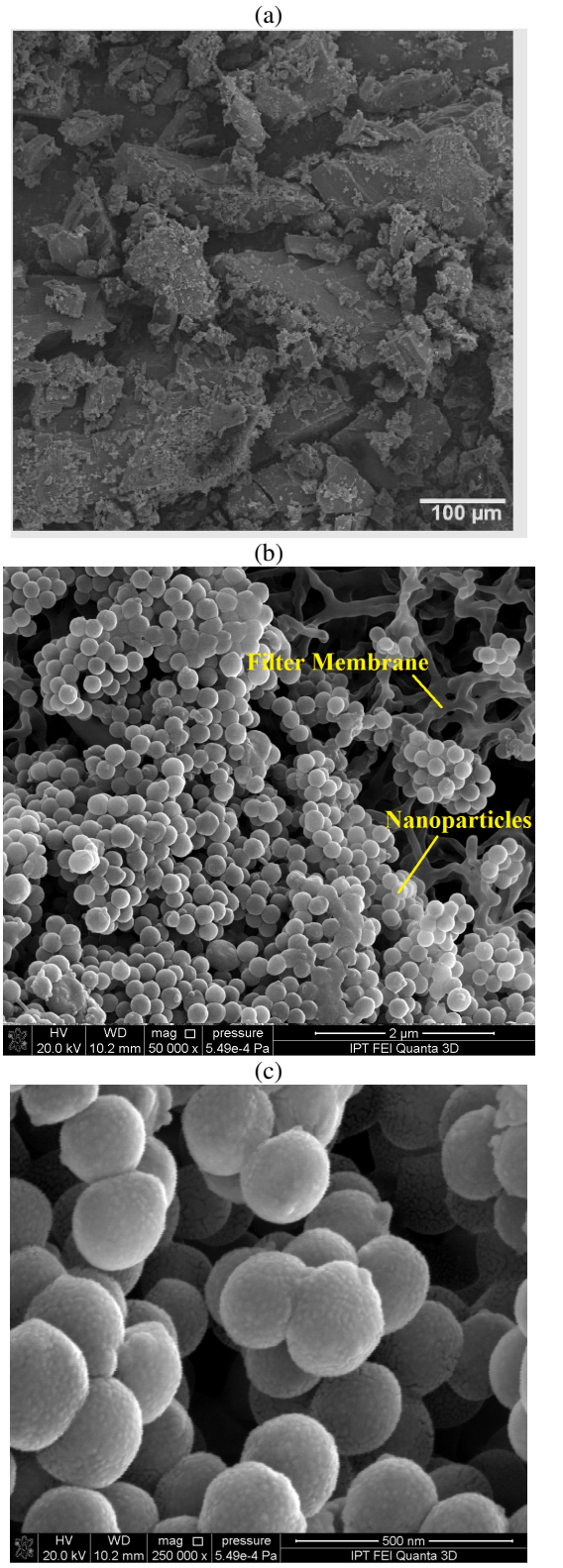


Figure 4. Observed Rifampicin nanoparticles: (a) Raw Rifampicin obtained by Dias. S [1] SEM, (b)Processed Rifampicin in 50.000 of zoom and (c) 250.000 FEG-SEM

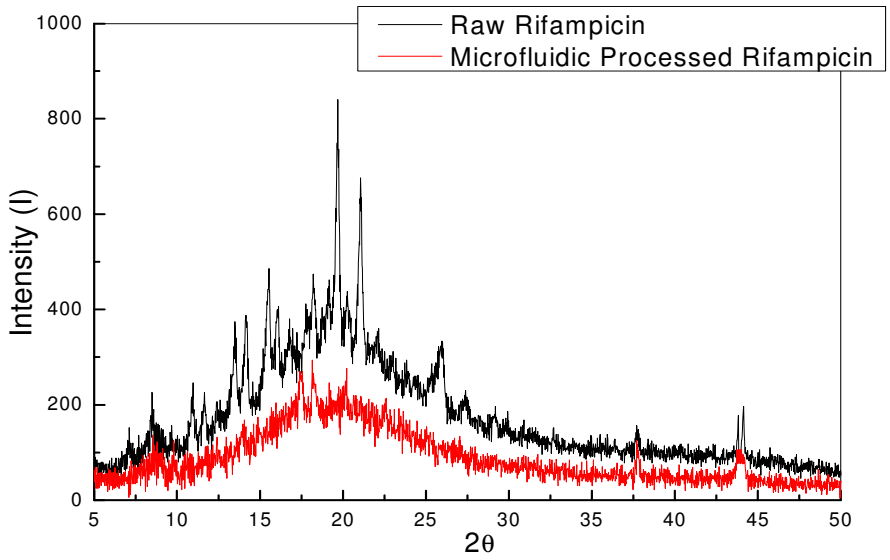


Figure 5: XRD profile of Raw Rifampicin and Processed Rifampicin.

CONCLUSIONS

In this work we have shown the results of Rifampicin nanosuspension obtained with microfluidic devices. The studies show the viability of obtaining the Rifampicin nanoparticles in a small size and low polidispersivity using microfluidic devices. The small size of the particle and the change in amorphous crystalline profile could improve the Rifampicin dissolution rate and bioavailability. FTIR, DSC and dissolution rate analysis are in progress.

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