Scaling up of Rifampicin Nanoprecipitation Process in Microfluidic Devices

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Abstract- **Microfluidic devices have become an important tool to produce micro and nanoparticles. However, the operation ranges of these systems are still a challenge when we think of large scale industrial applications. In this work we present two microfluidic devices for scaling up a nanoprecipitation process. The microfluidic systems are microfabricated in glass substrates and the flow distribution are done through reservoirs and a branching system, with four outputs in each device. In these systems we can operate in mL/min range and it is possible to have a yield up to ten times higher than a single channel system. We use the devices in a rifampicin nanoprecipitation process, obtaining nanoparticles in a range of 250 nm. As expected, parameters such as total flow rate and ratio between phases are determinant in the final mean particle size. Each output of our devices produces homogenous results and we can see that these results can be improved to obtain nanoparticles in larger volumes.**

Keywords- Nanoprecipitation; Microfluidic Device; Scale up; Rifampicin; Flow Focusing; Parallel Microchannels

I. INTRODUCTION

Microfluidic systems have been used for various applications such as the production of single [1] and double emulsions [2] and nanoparticles [3] with numerous advantages on size control, economy of reagents, among others [4, 5]. A relevant question, however, is that these systems operate with flow rates in a scale of μL/min and often take hours to produce a few milliliters of solution [6]. Thus, on one hand the systems are accurate to produce homogeneous particles with low consumption of reactants in a continuous flow, on the other hand, they have low productivity. The solution to this problem has been sought by producing a magnification systems with the microfabrication of parallel microfluidic systems on the same substrate [7, 8].

Multiply the number of channels in a single system maintaining the same transport characteristics obtained in a microfluidic system with a single channel is a critical issue. Moreover, the flow distribution should be designed to not require a greater number of fluid inlets, connectors, and pumps to maintain the flow. Some solutions are found for flow distribution such as the use of reservoirs [9], use of porous membranes or arrays [10], an external distribution head [11] and the use of systems with channels in several layers using the flow distribution by following Murray's Law [12, 13]. The use of reservoirs is an interesting strategy to avoid the use of more layers or external devices for flows distribution. The flows branching, using Murray's Law, presents a well-established method to distribute flows in a homogeneous way [14].

Several articles present these different strategies to scale up process such as single [15, 16] and multiple emulsions [17], cell capture [18], heat exchange [19], and in the same way we can amplify the nanoparticles production by anti-solvent nanoprecipitation. Tetradis-Meris et. al. [20] show a system with 180 microfluidic devices in PDMS. However, polymers do not withstand high pressures without deformations and are not suitable for using organic solvents normally used in nanoprecipitation process [17]. Glass substrates can support higher pressures than polymers and are suitable for processes with organic solvents. An important example for scaling up process is microfabricated in quartz and has 128 channels in the system and a stainless steel external head for flows distribution [11]. However, this system has several holes and a sophisticated external head which can not be easily microfabricated by anyone who does not have access to a laboratory with large microfabrication capability. In this way, we present a multi-channel system in glass substrates for rifampicin nanoprecipitation process, using two different strategies for flow distribution, considering a non-expansive microfabrication method.

Rifampicin ($C_{43}H_{58}N_4O_{12}$) is an antibiotic commonly used in Tuberculosis and Hansen disease treatments. However, rifampicin presents a large variability in its bioavailability, due mainly to its two polymorphic forms. Because of this crystalline characteristic, rifampicin shows variation in bioavailability in oral dosage form, hindering their use in tuberculosis treatment [21]. In the Biopharmaceutics Classification System (BCS) system, which presents an international classification of drugs bioavailability, rifampicin belongs to class II. Class II refers to drugs with low aqueous solubility and high permeability through the intestine. The World Health Organization requires that the formulations involving rifampicin bioavailability tests have proven to be indicated before [22].

Various efforts have been invested to improve bioavailability of rifampicin. In Son et. al. [23], for example, the crystal structure was modified through a hydration process being further processed in the Spray Dryer system for size reduction. The particle size was reduced from 42 micrometer (raw rifampicin) to 2 micrometer (Rifampicin and processed hydrated). Other examples such as amorphization [23], pH modification and particle size reduction [24] or rifampicin encapsulation in
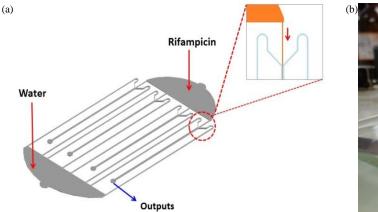
liposomes [25], biodegradable polymers [26] and hydrophilic agents [27], lactose microparticles [28] and antisolvent precipitation in batch process are also used [29].

Microfluidic devices have offered an important tool to improve bioavailability of great amount of drugs, by synthetizing homogeneous nanoparticles or nanocapsules in a self-assembly way [30, 31]. In this work we present the first studies related to the scaling up of the rifampicin nanoprecipitation process. In our previous work we observed the rifampicin nanoprecipitation in a single microfluidic system [32]. In this system we observed nanoparticles with sizes about 100 nm, with low polidispersity, with amorphous profile and better dissolution rate compared to raw rifampicin. We worked with a maximum water flow rate of 200 μL/min in a single channel system. Here we have improved the nanoparticle production process by scaling up the microfluidic system. We microfabricate systems in glass substrates with two different flow distribution configurations: a device with flow distribution using a reservoir and the other one with branch distribution. The use of reservoir is suitable for the microfabrication of planar systems and the branch systems require the use of more substrate layers, which leads to some difficulties in the sealing process.

II. EXPERIMENTAL SECTION

A. Microfluidic Devices Fabrication

We present two models for scaling up nanoprecipitation process. The first device uses a reservoir to distribute flows (Fig. 1, we show the scheme and the device in operation) and the second uses a branch distribution based on Murray's Law [33] (Fig. 2). We used microscope glass slides to obtain the microfluidic devices using standard photolithographic and wet etching procedures with hydrofluoridric acid solution. We adopt the UV glue bonding process to seal the channels with a cover glass and produce the microfluidic system with three layers. The devices is designed to have four outputs and the flow focusing geometries is microfabricated with central channel of 100 μm in width, side channels of 110 μm in width and depth of 85 μm. In Table 1 the dimensions of each device are detailed.
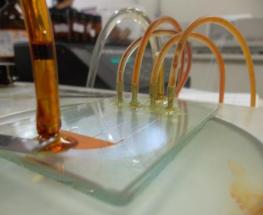

Fig. 1 Parallel microfluidic device with flows distribution by reservoirs: (a) scheme presenting the position of water and rifampicin reservoirs and (b) the microfluidic device microfabricated in glass in operation
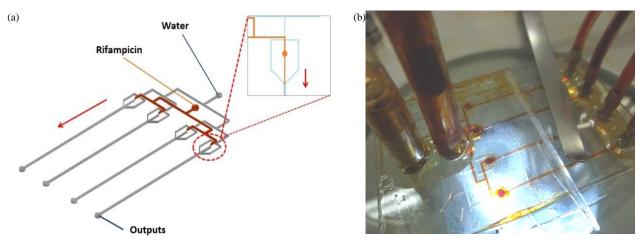

Fig. 2 Parallel microfluidic device with flows distribution by channels branching: (a) scheme of the device that follow the Murrays Law. This device was microfabricated with three glass layers and (b) the device in operation

TABLE 1 CHANNELS AND RESERVOIRS DIMENSIONS OF EACH DEVICE

Type of Device	Rifampicin Reservoir (Area mm^2)	Water Reservoir (Area mm^2)	Rifampicin channel width (μm)	Water channel width (μm)	System Depth (μm)
Reservoir	683 $\pm$ 1	723 $\pm$ 1	100 $\pm$ 1	110 $\pm$ 1	85 $\pm$ 1
	Water channel width 1 (μm)	Water channel width 2 (μm)	Water channel width 3 (μm)	Rifampicin channel width 1(μm)	Rifampicin channel width 2 (μm)
Branching	145 $\pm$ 1	126 $\pm$ 1	110 $\pm$ 1	115 $\pm$ 1	100 $\pm$ 1

B. Nanoprecipitation Process

Rifampicin is dissolved in methanol in a concentration of 50 mg/mL and the flow is described as Q_{RIF}. De-ionized water flow (Q_{WATER}) is used as anti-solvent and is introduced in the side microchannels. We do not use any kind of surfactant in these experiments. We evaluate different total flow rates supported by each device and we observe each output individually, comparing the results by particle sizes measurements. For a reservoir geometry device we operate the systems with flows of 600 μL/min up to 2000 μL/min and, for branch geometry we test flows of 500 up to 800 μL/min. We also observe the influence of the flow rate ratio R, which is defined as Q_{WATER}/Q_{RIF}, for each device. Syringe pumps, PHD 4400 (Harvard Aparatus), are used for controlling the fluid flows. The flow focusing behavior on the microchannel is observed using an optical microscope (Coleman, XTB-2T). The rifampicin nanoparticle size measurement is done in a Dynamic Light Scattering apparatus (Delsa^{TM} Nano C Particle Analyzer, Beckman Coulter). The rifampicin is maintained as a suspension and the DLS analyzer performed six measurements on each sample. The nanoparticles morphology is observed by FEG-SEM (Quanta 3D model, FEI). Rifampicin nanosuspension is filtered in a hydrophilic membrane with 0.22 μm in pore (Millipore Ind.) and is maintained in this membrane for FEG-SEM characterization. The membrane is fixed on the FEG-SEM stub using cooper double-sided adhesive tape and sputtered with 20 nm of Au/Pt coating. We compare the morphology of raw rifampicin with microfluidic processed rifampicin. The raw rifampicin has a particle size of 42 μm, and is diluted in water than filtered for SEM-FEG analysis.

III. RESULTS AND DISCUSSION

A. Particle Sizes

Fig. 3 show the results for the particle size measurement in reservoir microfluidic system in function of that the ratio between phases R for three different flows (600, 1400 and 2000 μL/min). The system can produce smaller nanoparticles when it is operated at higher flows (1400 and 2000 μL/min). In this system we can work with values of R of 20, without losing the flow focusing phenomenon. As expected, we can observe that with the increase of R we have a decrease in particle size for all flow rates used. The particle sizes vary from 250 nm up to 1.4 μm for the reservoir microfluidic system. The polidispersity indexes vary from 0.18 up to 0.3, yielding nanoparticles with low polidispersity index.
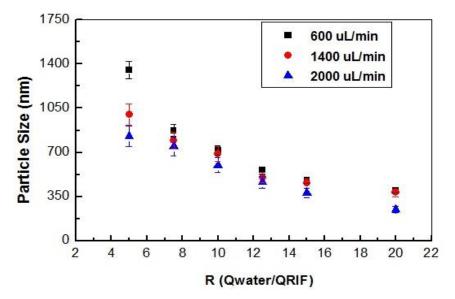

Fig. 3 Rifampicin particle size (nm) obtained in reservoir microfluidic device in terms of ratio
between phases R (Q_{water}/QRif) for three different flow rates: 600, 1400 and 2000 μL/min

In Fig. 4(a) we can see the picture of flow focusing on the reservoir system when the device operates with a flow of 2000 μL/min with R of 20, yielding a particle size of 250 nm as we can see in Figure 4(b).
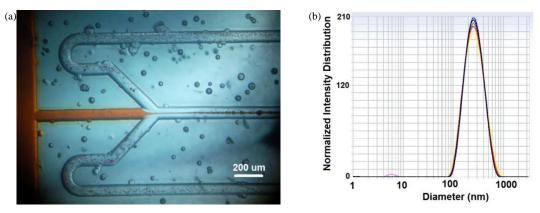
Fig. 4 Rifampicin flow focusing on reservoir microfluidic system: (a) detail of a flow focusing using ratio (R) between phases of 20 and a water flow of 2000 μL/min and in (b) the rifampicin particle size measurement by DLS of 250 nm obtained in this flows conditions

The same experiment is conducted in the microfluidic system with branching distribution flows, operating with flow rates of 500 and 800 μL/min. The flow rate 800 μL/min is the maximum fluid flow possible used in this system, which corresponds to 4 times the rate used in the single channel system designed in our laboratory (200 μL/min). In Fig. 5 we show the results of particle size measurement in function of R, for two fluid flows used 500 and 800 μL/min. The rifampicin particle sizes vary from 350 nm up to 1 μm, with polidispersity index below of 0.3 for high values of R, which yield the smallest sizes. The maximum possible adjustment of R in this system is 15.
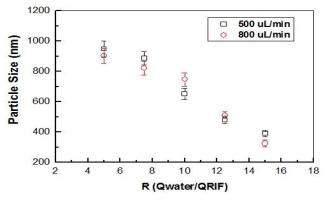

Fig. 5 Rifampicin particle size (nm) obtained in branching microfluidic device in terms of ratio between phases R (Q_{water}/QRif) for two different flow rates: 500 and 800 μL/min

In Fig. 6 we show the flow focusing on rifampicin using a flow rate of 800 μL/min and a ratio between phases of 15. In this case we have sizes of 350 nm as we can see in Figure 6(b) on the DLS measurement result.
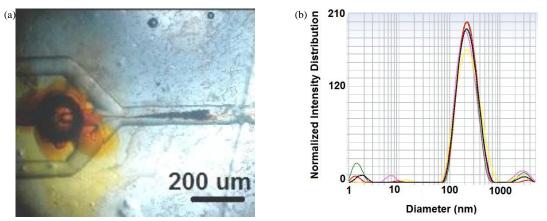

Fig. 6 Rifampicin flow focusing in branch microfluidic system: (a) detail of a flow focusing on using ratio (R) between phases of 15 and a water flow of 800 μL/min and in (b) the rifampicin particle size measurement by DLS of 350 nm obtained in this flows conditions

In our previous studies [32], we work with a water flow rate of 200 μL/min, and in this single system, it is possible to obtain nanoparticles in a range of 100 up to 800 nm, controlling the ratio between phases R up to 50. Observing these new systems, we can see that it is possible to increase the rifampicin production rate, by 4 up to 10 times, although the minimum particle size obtained in these parallel systems is 250 nm.

B. Evaluating Outputs

An important question is to know if each channel of the system produces the same nanoparticle size. In Fig. 7 we show the nanoparticle sizes of each channel of the reservoir system, using a fluid flow rate of 2000 μL/min for different values of R.
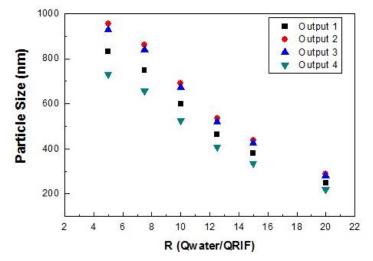

Fig. 7 Rifampicin particle size (nm) obtained in each output of reservoir microfluidic device in terms of ratio
between phases R (Q_{water}/QRif) for a water flow rate of 2000 μL/min

As we can see, when the values of R increase, the difference in the nanoparticle sizes between each output is reduced. The maximum difference in sizes is 30%, for low R values, and the minimum is 5% for high R values. However, all channels produce particle sizes in the same order of magnitude. Low R indicates that rifampicin flow do not have a thin flow focusing, resulting in large particles sizes, without homogeneity between each outputs. For high values of R, the rifampicin flow is focused by water flow, yielding a thin rifampicin flow surrounded by a large volume of water. In this case, the solvent diffuses to water rapidly, creating a supersaturation condition that allows more nucleation than particles growth, yielding particles in nanoscale range. In Fig. 8 we can see the results of each output for the branch microfluidic system. As we can see, the differences between each output are smaller than reservoir system and the results also become more homogeneous as R increases. The particle sizes of each output are in the same order of magnitude, with differences of no more than 22%.
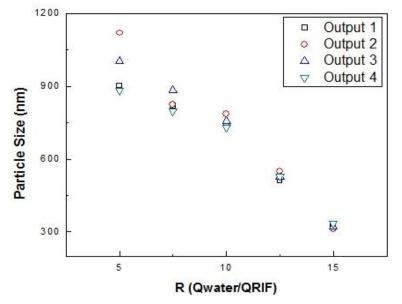

Fig. 8 Rifampicin particle size (nm) obtained in each output of branching microfluidic device in terms of ratio
between phases R (Q_{water}/QRif) for a water flow rate of 800 μL/min

C. Nanoparticles Morphology

We observe the morphology of Rifampicin nanoparticles using a Scanning Electron Microscope SEM-FEG, and the results are presented in Fig. 9. In Fig. 9 (a) we show raw rifampicin, without microfluidic process. As we can see in Fig. 9 (b) the particles have a round profile and this fact, together with particle size reduction, can improve the dissolution rate of this hydrophobic drug. The sample is obtained in the reservoir microfluidic device, using the flow rate of 2000 μL/min.
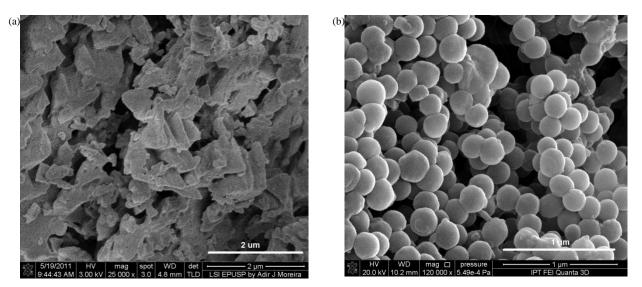
Fig. 9 Rifampicin micrographs obtained by SEM-FEG technique: (a) raw rifampicin without microfluidic process and (b) the rifampicin nanoparticles obtained in the reservoir device, using a flow rate of 2000 μL/min

IV. CONCLUSIONS

We observe the nanoprecipitation process in two microfluidic devices designed to scale up the nanoprecipitation process, with four outputs each system. These systems yield rifampicin nanoparticles with sizes in a range of 250 nm up to 1.5 μm, with low polidispersity in the cases that we use high values of R. It is important to note that these experiments are done without the presence of surfactants. We can increase the nanoparticles production practically ten times, compared to the commonly flows ranges used in our laboratory (200 μL/min). The reservoir geometry offer the advantage of not requiring more layers for distribution of flows, however, comparing each output, this system presents higher differences between particle sizes obtained in these outputs. In the best results we obtain nanoparticles with 250 nm of size using a flow of 2000 μL/min. The branch geometry presents more homogeneity between particles sizes, although there are difficulties in microfabrication process and the maximum operation flow range is 800 μL/min. The devices need some adjustments not only in geometry, but also in microfabrication process. However, the synthesis of nanoparticles in a parallel system is possible and can be improved to obtain higher volume flow.

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