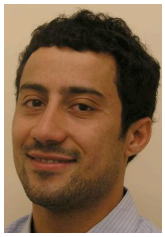
O2-3

Microfluidic production of polymeric micelles for mithramycin encapsulation

Capretto L,[1#] Hill M,[1*] Zhang X,[1*] Mazzitelli S[2] and Nastruzzi C[3]
[1] School of Engineering Sciences, University of Southampton, Southampton SO17 1BJ, UK
[2] Department of Biochemistry and Molecular Biology, University of Ferrara, Italy.
[3] Department of Chemistry and Technology of Drugs, University of Perugia, Italy.
* Supervisor # Contact e-mail: lc4g08@soton.ac.uk



INTRODUCTION AND OBJECTIVES

Microfluidic-based reactors offer a series of potential advantages that can be leverage to control the reaction environment in order to produce material of required features in term of size and size distribution (deMello 2004). In microfluidic reactor polymeric micelles (PMs) can be synthesized through a nanoprecipitation process upon rapidly mixing a polymeric solution with anti-solvent. The solvent jump, provided by the rapid mixing obtained in the microfluidic device, triggers the precipitation of the block copolymer unimers and their aggregation to form PMs.

The current paper describes the production of polymeric micelles using Pluronic® F127 by microfluidic-based nanoprecipitation. The effects on the nanoparticle size and size distribution have been examined systematically by varying polymer concentration, flow rate ratio of solvent to non-solvent, and the microchannel dimension. The mixing process has been further analyzed by a computational fluid dynamics (CFD) model in order to understand the hydrodynamics and diffusive mixing process that leads to the PMs formation. Following the production of Pluronic PMs, we then investigated the use of microfluidic-based nanoprecipitation to assemble mithramycin drug-encapsulated PMs. Mithramycin have gained increasing attention as a potential therapeutic agent for haematological disorders, including β-thalassemia and sickle cell anemia. Mithramycin therapeutic effect steams from its capability to induce the in-vitro erythroid differentiation of human leukaemic K562 cell line that is associated with an increased expression of embryo-fetal globin genes (Fibach 2003). The encapsulation process of mithramycin within the PMs core has been investigated in a statistical fashion in order to understand the effect of different production parameters, such as drug concentration and flow rate ratio, on the encapsulation efficiency and dimension of the produced PMs. Finally the produced micelles were analysed for antiproliferative and differentiation activity, in vitro, on K562 cells.

MATERIALS AND METHODS

The microreactors were fabricated in glass using a classical glass wet etching protocol (McCreedy 2001) and had an approximately semi-circular cross-section with the dimensions summarized in Table 1 for the three microreactors used.

Table 1 : Microfluidic reactor dimensions and operating set-ups.

Microreactors	1	2	3
h (µm)	53	29	17
w_o (µm)	130	80	57
w_b (µm)	24	22	23
Total flow rate (mL/h)	2.00	0.65	0.30

For the production of PMs the following chemicals were used: Pluronic F127® (kindly donated from BASF, Germany), DMSO (Sigma-Aldrich, USA) and mithramycin complex (Sigma-Aldrich, USA). In microreactors PMs were prepared using a flow focusing enhanced microfluidic mixer. The hydrodynamic flow focusing was created by three flow streams in parallel where the middle stream was the solution of Pluronic F127® in DMSO and on each side was a deionised water stream. Nanoprecipitation by hydrodynamic flow focusing was carried out using a total flow rate (polymer stream flow plus two deionised water stream flow) of 2.00, 0.65 and 0.30 mL/h for Microreactors 1, 2 and 3, respectively. After the preparation of PMs, water was added to the samples in order to have a constant amount of solvent (3.2 % of volume) among the different samples. To obtain the hydrodynamic particle diameter of the produced PMs, a nanoparticle analysis (NTA) system Nanosight LM10 (Nanosight Ltd. Amesbury, UK) was used. Computational fluid dynamyc simulations based on the finite element method (FEM) were performed utilizing Ansys Fluent 12.1.4

RESULTS AND DISCUSSION

Mixing is a key step in the nanoprecipitation process that can strongly affects the size characteristics of the produced PMs. In the laminar flow regime at low Reynolds numbers mixing generally occurs as a result of diffusion. We can estimate the time needed for a complete mixing by diffusion (τ_{mix}) for hydrodynamic flow focusing using a two-dimensional model

$$\tau_{mix} \approx \frac{w_f^2}{4D} \approx \frac{\left(w_b + \frac{\pi}{2}h\right)^2}{4D\left(1+\frac{1}{R}\right)^2}$$

where D is the diffusion coefficient of the solvent. R is the flow rate ratio of the focused stream to the total flow rate of the sheath flow and w_b and h are the bottom width and height, respectively, of the main channel (Table 1). According to Eq. (1), the mixing time for solvent ex-

change can be controlled by varying both the flow rate ratio (R) and microchannel dimension.

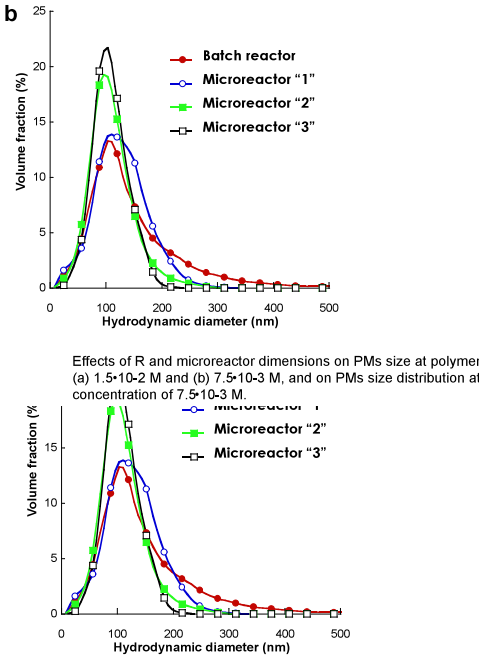


Figure 1 : Effects of microreactors dimension and flow rate ratio (R) on PMs size (a) and size distribution (b and c).

As can be seen from the results depicted in Fig. 1, a decrease in R led to the formation of smaller (Fig. 1a) and more uniform (Fig. 1c) PMs, indicating the role of the mixing time in controlling the dimensional characteristic of the produced PMs. In addition, an increase of the polymer initial concentration leads to the formation of larger PMs (data not shown). At a given R value the PMs mean diameter and polydispersity (Fig. 1b) decreased when the microchannel dimension was reduced (Fig 1a), according with a decrease of the mixing time as predicted from eq. 1. Comparing microfluidic reactors with batch systems, it was found that PMs obtained using a microreactor was generally smaller and more uniform in size than that using a batch reactor (Fig. 1a and b). Fig. 2a shows the variation of the mean size of PMs as a function of width of the focused stream obtained within Microreactors 2 and 3, respectively. According to equation 2 the width of the focused stream is directly connected with the diffusive mixing time. A linear relationship between PMs mean size and width of the focused stream was found for both microreactors. However, the difference between the two straight lines of the two microreactors was indicative that the width of the focused stream was not the only parameter that affected the PMs size. Figure 2b shows the variation of the mass fraction profile, as computed by CFD study, along the mixing channel of microreactor "2" and "3" for *R* equal to 0.05 and 0.1, respectively. With *R* being set as reported above we were able to obtain a comparable focused stream in the two microreactors. The variation of the mass fraction profile along the channel for the two microreactors shows that the mixing proceeds slower in microreactor "3" than in microreactor "2".

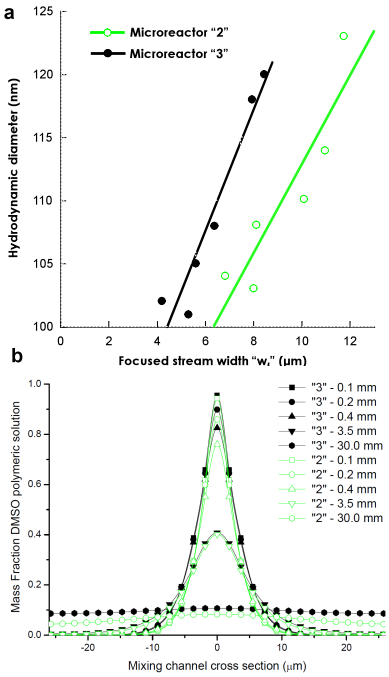


Figure 2 : Effect of width of focused stream on PMs mean size for reactor 2 and 3 (a) and CFD simulated mixing of DMSO along microreactor 2 and 3 (b).

This effect was likely associated with the variation of the amount of polymeric solution introduced in the microreactors that caused the diffusivity of the species to increase. Finally we prepared mithramicyn loaded PMs. The produced PMs were analysed for antiproliferative and differentiation activity in vitro on K562 cells. The results obtained show that microfluidic based strategy can be conveniently applied for the encapsulation of mithramicyn in PMs. Furthermore, the produced micelles were able to deliver their payload to K562 cells triggering their in-vitro erythroid differentiation.

CONCLUSION

This study demonstrates that microfluidics is a powerful technology for microfluidic nanoprecipitation-based production of drug loaded PMs as compared to batch systems since it enables better control, reproducibility, and homogeneity of the size characteristics of the produced PMs. In addition the study demonstrate that mithramicyn loaded PMs were able to trigger the K562 cell erythroid differentiation.

REFERENCES

• J. C. deMello et al. (2004) *Microsacle reactors: nanoscale products*. Lab Chip. 4 11.
• E. Fibach et al. (2003) *Mithramycin induces fetal hemoglobin production in normal and thalassemic human erythroid precursor cells*. Hematopoiesis 102 (4) 1276.
• T. McCreedy (2001) *Rapid prorotyping of glass and PDMS microstructure for micro total anlytical system and micro chemical reactors by microfabrication in the general laboratory*. Anal. Chim. Acta. 427 39.