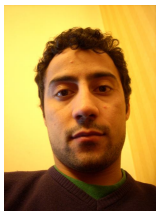
# Production and optimization of SLM by flow-focusing technology



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## Introduction

Microfluidics is a "micro" technological field dealing with the handling of fluids, having applications in different fields, including chemical processing, food manufacturing, pharmaceutic, biotechnology and cosmetic. In recent years the microfluidic devices are conveniently applied as new tools for the formation of multiphasic regimes of flow (C.N. Baroud. et al. 2004), later converted, in different ways in highly monodiperse spherical polymeric microparticles (K. Liu et al. 2006).
The chemical nature of the droplet phase (disperse phase) determines the next step in which the droplets are transformed into microparticles by a consolidation procedure. Droplets containing monomers can be solidified by means of thermally initiated (S. Sugiura. et al. 2002) or UV-initiated (T. Nisisako et al. 2004) polymerization. Alternatively, droplets of polymer dispersions can be hardened by different procedures including solvent evaporation (M. Seo et al. 2005), chemical reactions (I. Cohen et al. 2001) or ionic cross linking (K. S. Huang et al. 2006).
Lipid microspheres also called lipospheres (LS), are a new type of fat based encapsulation systems developed for drug delivery of bioactive compounds (especially lipophilic compounds). LS combine the advantage of polymeric nanoparticles; fat emulsion and liposomes avoid some of their disadvantages such as cytotoxic effect after they fagocytosis, toxic effect of the organic residual after the production of polymers, lack of large industrial scale production (R. Cortesi et al. 2005).
The present paper reports the design, preparation and characterization of SLM by a flow focusing technique based on a novel microfluidic device. In addition, a factorial design study (L. Eriksson et al. 2000) was conducted to analyze in which extent the preparation parameters influence the dimensional and morphological characteristics of the produced lipophilic microparticles.

### Material and method

Glyceryl tripalmitate (GTP) was obtained from Fluka Chemical Co (Buchs, Switzerland), glyceryl monostearate (GMS) was from Gattefossé (Saint-Priest Cedex, France), polyvinyl alcohol Celvol205® (PVA) was from Celanese chemicals Europe GmbH (Kronberg, Germany).

**Fig. 1. The flow focusing device LB-EXT/1 used for the preparation of solid lipid microparticles**

The production strategy is based on the formation of a multiphase flow by a co-axial flow microdevice LB-EXT/1 (Fig. 1) (L. Bilancetti et al. 2006).
The main parts of the microdevice are: (a) a pressurized and thermostated reservoir, filled with melted lipids, connected to microdevice (b) the body of the microdevice (constituted of derlin) with

an internal diameter of variable size (in which the water phase is pumped) and (c) a variable-gauge steel needle (in which the melted lipid phase is pumped). The needle and outer tubule were coaxially positioned.
As an alternative of the pressurized and thermostated reservoir, the melted lipid phase can be pumped into the flow focousing device, by a syringe pump (KDS Model 100 Series, Kd Scientific) equipped with an easy temperature control system for syringe pumps (L. Capretto et al. 2008). The oil and water phases were pumped into the microfluidic devices by sylicon or teflon tubes. As internal oil phase, a GTP and GMS mixture (80:20) (oil phase, OP), melted at 70 °C, was used, that is slowly injected into the inner channel of the device. The second immiscible liquid (Water Phase, WP) was injected into the outer channel as continuous phase. Lipid mixture was forced into the focusing devices to form a multiphasic flow (droplets) represented by an o/w emulsion. Finally, the lipid microdroplets were solidified by collecting them into to a bath constituted of cold water in order to produce the final consolidated microbeads (Fig. 2).

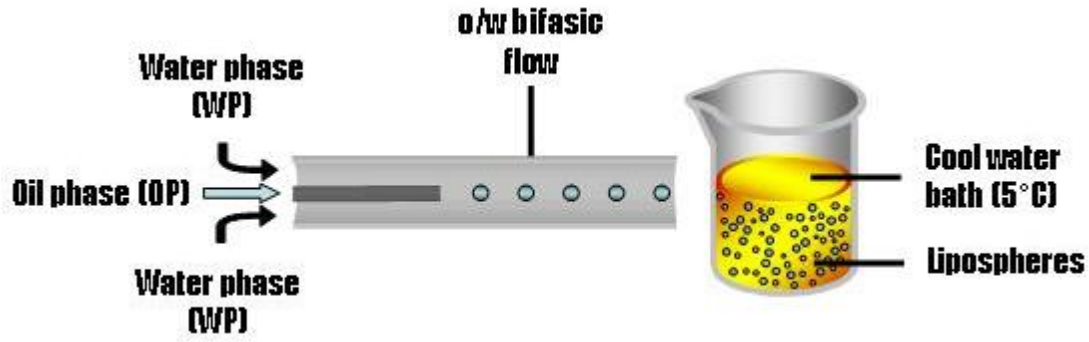


**Table 1. Effect of WP pumping rate on the size and size distribution of lipospheres produced by flow-focusing technology.**

The SLM were characterized in term of size, size distribution, morphology and surface characteristics by optical microscopy (Nikon SMZ 1500 stereo microscope, Tokio, Japan) and their size and size distribution (by number) were determined by photomicrograph analyses (EclipseNet version 1.16.5, Laboratory Imaging s.r.o. for Nikon B.V.).
The factorial design and the evaluation of the experiments were performed by the PC software MODDE 8.0 (Umetrics AB, Umeå, Sweden).

**Results and Discussion**

Initially we tested the possibility to produce SLM by flow focusing technique. To this aim, we designed a new focusing device by AUTOCAD 2004 software (autodesck, San Rafael, CA, USA). The first experiments were conducted by a "changing one separate factor a time approach" (COST), considering the following experimental parameters: the oil and water phase type and flow rate, the internal needle dimension, the stabilizer type and concentration, the tubing length and position. By the analysis of this first set of experiments we selected the three main (in term of statistical relevance) parameters, to a further analysis by "design of experiment approach" (DoE), considering: (a) the weight ratio between the two lipophilic constituent of SLN (namely, glyceryl monostearate and glyceryl tripalmitate), (b) the stabilizer concentration (PVA) and finally (c) the flow rate of the water phase.
The DoE approach represents a statistical investigation in which the effects of multiple factors are investigated simultaneously.
The results of the DoE analysis demonstrated that the parameter having the greatest influence on the SLM size and size distribution is the water phase flow rate, while the stabilizer concentration resulted to be the factor with the lower impact on the SLM size and morphology.

| Batch | WP pumping rate (mL/min) | OP pumping rate (mL/min) | Mean diameter ± SD (µm) |
|---|---|---|---|
| Lipff 1 | 8.13 | 0.15 | 1109.05 ± 162.24 |
| Lipff 2 | 9.06 | 0.15 | 810.44 ± 31.00 |
| Lipff 3 | 12.20 | 0.15 | 670.43 ± 34.96 |
| Lipff 4 | 16.26 | 0.15 | 483.15 ± 39.82 |

**Table 1. Effect of WP pumping rate on the size and SD of lipospheres produced by microfluidic device.**

By examining the sterephotomicrograph reported in Fig. 3 and the results reported in Tab. 1, it is evident that is possible to prepare SLM with a broad range of mean diameter, preserving good narrow size distribution and morphological characteristics.

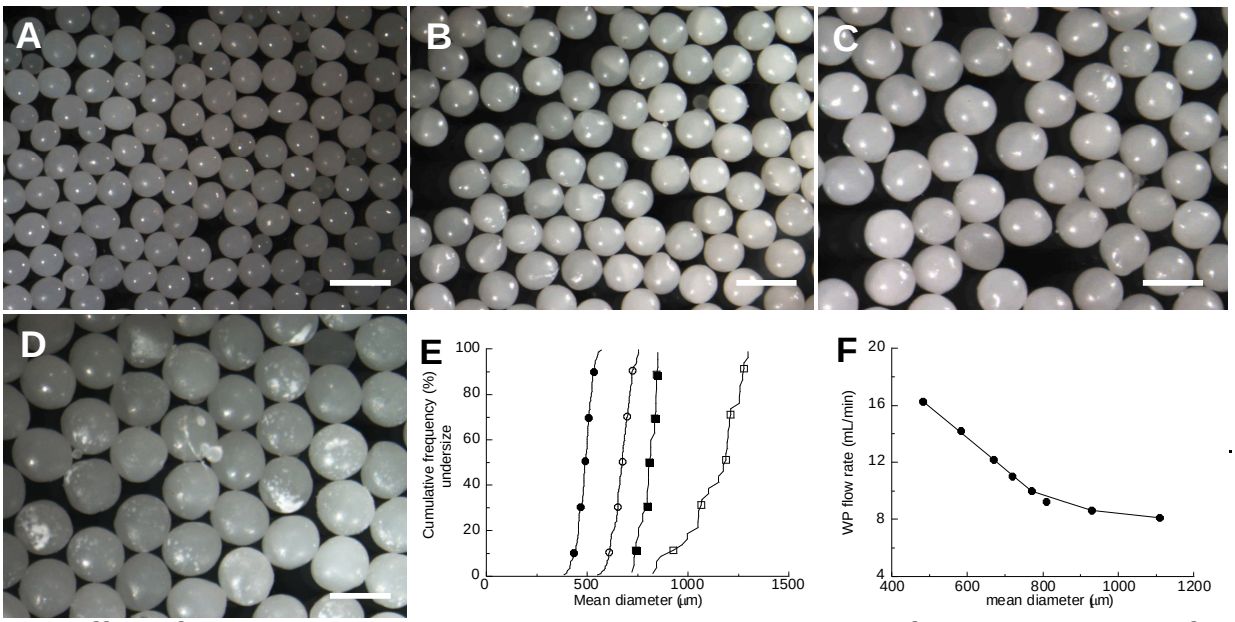


**Fig. 3. Effect of WP pumping rate on the size and size distribution of SLM produced by flow-focusing technology. Stereo microphotographs of batches lipff1 (A), lipff2 (B), lipff3 (C), lipff4 (D). The bars correspond to 1000 µm. Cumulative frequency distribution plots of lipff1 (solid circles), lipff2 (open circles), lipff3 (solid squares), lipff4 (open squares) (E). Plot of the effect of the WP/OP flow ratio on mean diameter of produced lipospheres (F).**

More importanly, the SLM size can be easily adjusted by changing the flow regimens in the microchannels. In this respect, Fig. 3F shows the relationship between the flow rate (expressed as the WP to OP ratio) and the lipospheres dimensions (mean diameter). For instance, if the flow rate of the OP is maintained fixed at 0.15 mL/min, it can be observed that the lipospheres size decreases with the increase of the flow rate of the WP (in an almost linear fashion). Conversely, for a given fixed flow rate of the WP (continuous phase) the emulsion droplet size increases and as a consequence the final dimensions of the SLM are larger (data not show).

## Conclusion

This paper confirms that microfluidic methods appear to be one of the most effective procedures for the production of lipospheres with an extremely narrow size distribution. The results obtained by the factorial design approach, applied to the optimization and the screening of the experimental parameters, clearly shown that the parameter influencing more deeply the mean particle size and distribution is the oil phase/water phase flow ratio.

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