Flow Focusing: A Versatile Technology to Produce Size-Controlled and Specific-Morphology Microparticles**

Lucía Martín-Banderas, María Flores-Mosquera, Pascual Riesco-Chueca,
*Alfonso Rodríguez-Gil, Ángel Cebolla, Sebastián Chávez, and Alfonso M. Gañán-Calvo**

The Flow Focusing platform is especially advantageous for micro and nanoparticle production. This versatile technique is amenable to designing the size, surface treatment and internal topology of the particles; mechanical stresses are minimal—an optimal feature for the manipulation of delicate substances. Multiplexing and high-rate production are readily implemented. Adaptive operational design can lead, in one single step, to finely tuned microcapsules encasing different products within a targeted morphology. This achievement is of great significance for most microcapsule applications in the biosciences (for example, drug delivery, cell encapsulation, and the production of bead arrays).

Keywords:
· drug delivery
· fluorescent probes
· microencapsulation
· microfluidics
· microparticles

Single- and multiple-core microcapsules are increasingly attractive owing to potential applications in biochemistry, biomedicine, pharmaceutics, and environmental science.[1] Since 1994, we have developed a technological platform for fluidic manipulation and particle microdesign called "Flow Focusing" (FF).[2] We describe here some innovative FF applications for micro- and nanoparticle production. Salient features of the technology are: a) FF is compatible with different fluid combinations (liquid–liquid, liquid–gas) using simple liquids, polymeric solutions, emulsions, suspensions, or melted solids; b) target-size microparticles, with a narrow size distribution, are obtained in just one step, with no external excitation source, and without additional purification steps; c) FF produces smaller particles than most other technologies, where particle size is determined by the nozzle dimension; d) due to the special flow geometry, the particle-generating fluid is scarcely stressed, therefore FF is remarkably adequate for the encapsulation of labile compounds (proteins, cells, and similar entities).; e) FF is suitable for particle design, involving freely-chosen morphology, surface treatment, and composition (e.g., homogeneous particles, two-phase capsules, or hollow capsules); f) FF leads to extraordinarily high particle rates per orifice, and moreover, its microfluidic topology can be consistently and robustly up-scaled into two-dimensional arrays for the large-scale production of microspheres.

Flow focusing results from combining hydrodynamic forces with a specific geometry.[3] A FF device (Figure 1a) consists of a pressure chamber pressurized with a continuous focusing fluid supply (1). Inside, a focused fluid (2) is injected through a capillary feed tube whose extremity opens up in front of a small orifice linking the chamber with the exterior ambient. The focusing fluid stream (1) molds the fluid meniscus (3) into a cusp giving rise to a micro- or nanojet exiting the chamber through the orifice; the jet diameter is much smaller than the exit orifice diameter, thus precluding any contact. Capillary instability breaks up the stationary jet into homogeneous droplets.[4] The feed tube may be composed of two or more concentric needles and

[*] L. Martín-Banderas, Dr. P. Riesco-Chueca, Prof. A. M. Gañán-Calvo
Dpto. Mecánica de Fluidos, Escuela Superior de Ingenieros
Universidad de Sevilla
Camino de los Descubrimientos s/n
41092 Sevilla (Spain)
Fax: (+34) 954487247
E-mail: amgc@us.es

A. Rodríguez-Gil, Dr. S. Chávez
Dpto. Genética, Facultad de Biología, Universidad de Sevilla
Profesor García González s/n, 41012 Sevilla (Spain)

Dr. M. Flores-Mosquera, Dr. Á. Cebolla
Ingeniatrics Tecnologías S.L.
Avda. Américo Vespucio 5
41092 Sevilla (Spain)

Supporting information for this article is available on the WWW under http://www.small-journal.com or from the author.
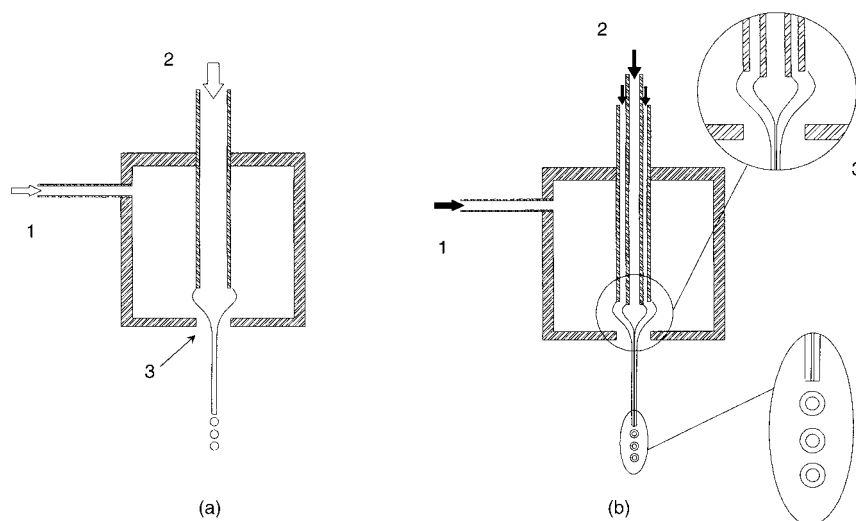
Figure 1. Flow-focusing atomizer. a) Simple jet: 1) focusing fluid, 2) focused fluid, 3) meniscus; b) compound atomizer with two concentric needles: 1) focusing fluid, 2) focused fluids: core fluid and shell fluid, 3) compound meniscus.

different liquids can be injected, thus leading to multilayer microcapsules with multiple shells of controllable thickness (Figure 1b). In short, FF ensures an extremely fast production of up to millions of droplets per second as the jet breaks up,[5] a rate much higher than that obtained by other microdripping techniques.

A low-Reynolds microfluidic or planar device (where the Reynolds number (Re) expresses the ratio of inertial forces to viscous forces) based on the FF principle has already been successfully utilized[6] to yield highly monodisperse objects with a nonspherical shape, where the break-up mechanism is controlled by the surface tension and the viscous stresses, while the geometry of the outlet channel determines the eventual shape of the particles. In contrast to such systems, the FF applications described below enjoy superior productivity owing to their high- and moderate-Reynolds operation. This causes a break-up pattern where inertia, surface tension, and geometry play the leading roles: a remarkable degree of size-control is achieved, ranging up to the lowest microscales. High-Reynolds applications are able to "tame" inertia, a potentially unruly component of the flow pattern, by the judicious use of design; thus they prove particularly suitable for miniaturization, given their sensitive and robust response to geometric fine-tuning. Moreover, the operation of such devices is strikingly energy-efficient, owing to the restrained role of viscosity.

Ensuring monodispersity in high-Reynolds FF requires controlling the Weber number, which is a measure of the relative focusing to focused inertia against the surface tension forces of the jet. When the focusing inertia per unit mass is very high (as in the case of using a gas) compared to that of a focused liquid, beyond a given threshold,[2] around We = 40, the breakup becomes turbulent, and monodispersity is lost. It is worth noting that liquid–liquid FF is considerably less restrictive, with both the jet and the outer stream running at a similar velocity, so that capillary forces are dominant even at high Reynolds numbers.

An additional feature of the applications described is their geometric simplicity, which is readily amenable to 2D-upscaling by bundling together an array of feed tubes. The microfluidic alternative[6] is more restrictive in that only 1D-upscaling (by piling up the channel planes) can be considered.

Next we describe some FF applications for microparticle generation. To illustrate the wide range of applications available depending on our design objectives, different fluid combinations have been chosen, as well as different nozzle geometries.

1. *Drug encapsulation*: An attractive application is biomedical drug encapsulation. Traditional methods such as conventional spray-drying or emulsion evaporation do not produce particles with a narrowly targeted drug content, especially when dealing with highly water-soluble drugs; monodisperse particle spectra are only obtained at the cost of a complementary treatment by means of a filtration or sieve system. In addition, the inner-particle morphology is highly influential on the drug-delivery profile.[7] Being able to design particles with a finely tuned size and internal topology to guarantee a specific delivery pattern, and achieving that goal in just one step, would prove a major achievement.

Thus, we encapsulated a hydrophilic antibiotic (gentamycin sulphate, GS) with a lipophilic biodegradable polymer poly(D,L-lactic acid-*co*-glycolic acid) (PLGA). We nebulized a water/oil (w/o) emulsion of an aqueous drug solution and an organic PLGA solution; air was chosen as the focusing fluid. Particles were collected as a dry powder at the bottom of a thermostated chamber. Using just one nozzle, and with diverse flow-rate ratios, 8 to 30 μm particles were obtained. Experimental data agree with the theoretical FF prediction (Figure 2). Alternatively, a concentric nozzle may be used; an antibiotic aqueous solution and an organic PLGA solution were respectively injected through the inner and outer passageway of a concentric capillary (Figure 1b). The resulting capsules consist of an antibiotic core encased by a PLGA shell. Table 1 illustrates the application of this technique to produce microparticles and microcapsules: a GS load above 10% was recorded, quite superior to previous achievements in the field of gentamycin encapsulation.[8]

A liquid–gas configuration was chosen to produce solid particles by means of a reverse thermostatic effect. We have designed a thermostated FF device for simultaneous warming of both fluids before their mixture. A biocompatible material (Gelucire 50/02, m.p. 46–51 °C) is introduced in a ther-

Table 1. Mean diameter and drug loading of gentamycin sulphate/ PLGA microparticles and microcapsules.

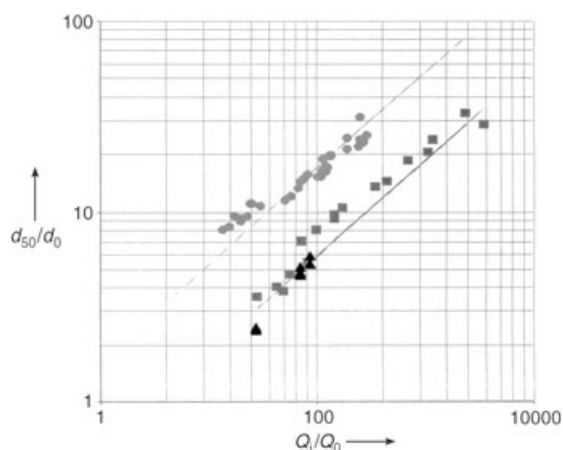|	Mean diameter [µm]	Entrapment [%]	Encapsulation efficiency [%]
Particles	20.51 ± 7.18	14.2	42.7
	11.58 ± 3.48	20.6	61.9
	6.69 ± 1.22	10.9	42.7
Capsules	13.94 ± 4.06	30.6	85.8
	6.54 ± 1.86	17.5	49.0

Figure 2. Theoretical FF prediction (full line) compared with experimental data. Particles are Gelucire (●), gentamycin sulphate/PLGA (▲), and polystyrene (■). For the latter two particles, solvent evaporation has been taken into account.

mostated container at 60 °C, pressure-fed into the capillary tube, and focused by the warm gas. When the steady ligament breaks up as it exits the device, droplet solidification is observed, with the external temperature lying below the polymer melting point. In the explored range of flow-rate ratios, 6 to 38 µm microparticles were obtained (Figure 2).

2. *Dye-labeled particles*. In research and diagnosis applications, dye-labeled fluorescent particles provide an excellent means for high-throughput analysis of biological molecules.[9] Despite all the efforts devoted to the production of dye-functionalized polymeric beads,[10] a straightforward methodology allowing massive preparation of fluorescent encoded microparticles with a uniform shape, homogeneous distribution, and controlled fluorescent properties would be welcome.

We report the preparation of dye-labeled polymeric particles by a modified emulsion/solvent evaporation/extraction encapsulation technique. A FF nozzle was immersed in a continuous phase of the emulsion. Using the liquid–liquid configuration, the oil drops of the disperse phase were generated without any shape deformation. To that end, we used a homogeneous solution of polystyrene (PS; 1–8 % w/v of PS with different M_w values) and a fluorophore (fluorescein, rhodamine B, or Nile Blue A) in EtOAc or CH_2Cl_2 as the oil phase, and distilled water as the focusing fluid (Figure 2).

Figure 3 shows 5 µm fluorescent particles obtained with the same operating conditions. Spherical, non-aggregated and nonporous particles with a very reproducible size distribution are produced, with freely-chosen and discernible fluorescent properties being guaranteed. As summarized in Figure 4, fluorescence was evaluated as a function of particle size and fluorescent probe content/type.

3. *Multiple-core particles*. We also investigated in some detail the evolution of a concentric stream involving three immiscible liquids forced through a small orifice.[11] Our selection of the surface-tension coefficients between the three phases ensures the robust production of particles with a multiple-core morphology, the number of cores being a freely chosen parameter (Figure 5).

In summary, flow focusing relies on a flow pattern that is certain to be very advantageous for efficient encapsulation and particle design. The salient features of FF are: 1) it can be used with a broad range of materials (simple liquids, solutions, emulsions, suspensions); 2) it lends itself to a simple particle-size control in the micro- and nanometer
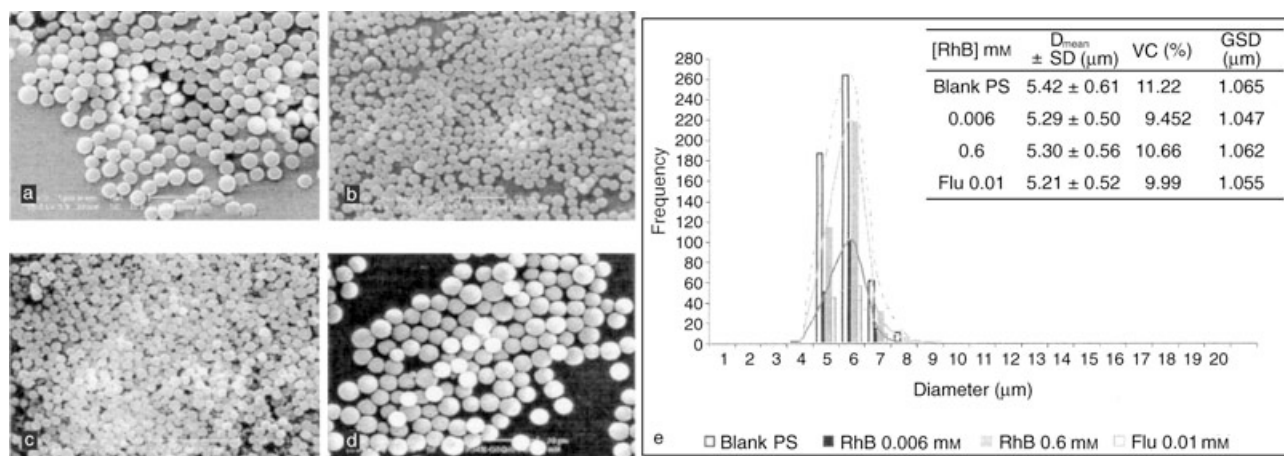

Figure 3. SEM images of freeze-dried 5 µm microparticles; a) blank PS; b) rhodamine B (0.6 mм); c) rhodamine B (0.006 mм); d) fluorescein (0.01 mм); e) particle-size distribution of the above samples.
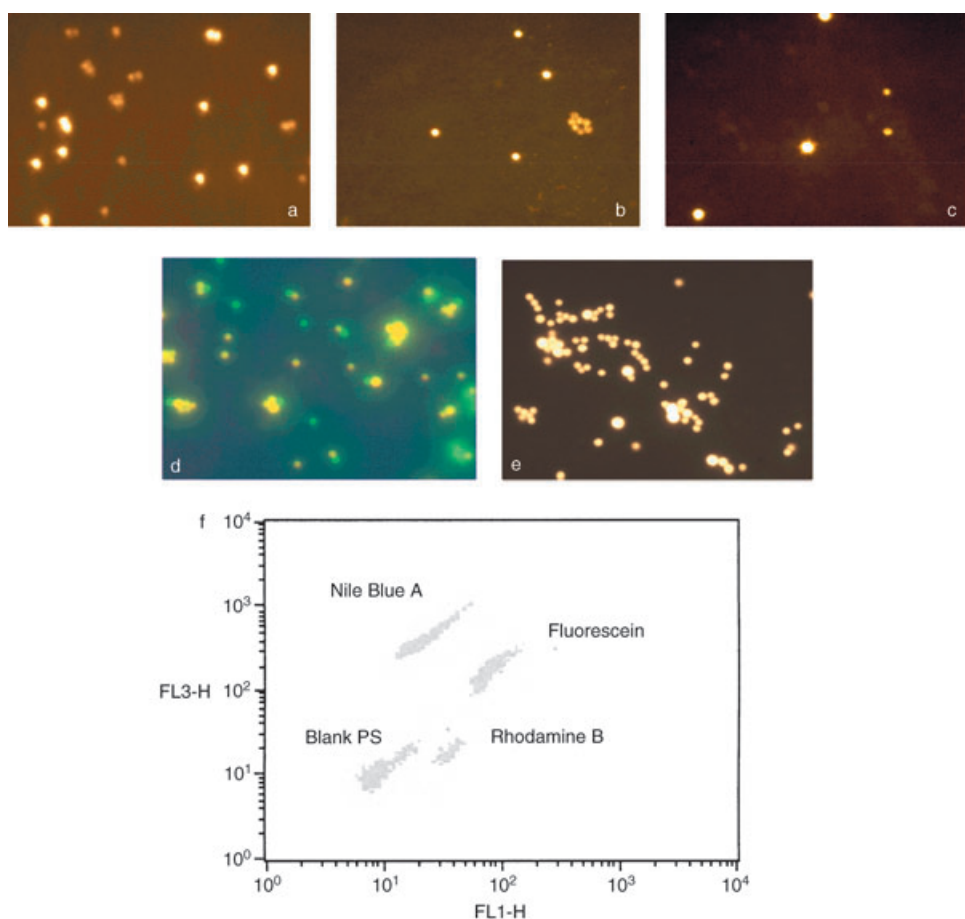
Figure 4. The discrimination of different microparticle populations: a–c) as a function of fluorescent probe content (rhodamine B, 5 μm); a) 0.006 mm/0.6 mm, b) 0.06 mm/0.6 mm, c) 0.006 mm/0.06 mm; d) as a function of fluorescent probe type (5 μm): rhodamine B 0.06 mm/fluorescein 1 mm; e) as a function of size (rhodamine B 0.6 mm): 5 μm/9 μm; f) dot plots showing profiles of fluorescent microparticle populations resulting from the analysis of a mixed sample in a flow cytometer.

design; 3) it uses gentle operating conditions that allow the encapsulation of labile molecules or microorganisms; 4) the technology has been scaled up by replicating the FF nozzle into a 2D-array structure.[12]

Herein, we have overviewed this novel and low-cost method for the straightforward production of controlled-morphology micro- and nanoparticles. FF lends itself to particle topology design and surface treatment; process variables include injection configuration, solidification procedure, choice of the fluid materials, and their flow rates. An adequate selection of the operational and design variables opens the door to morphology and geometry control (e.g., shell thickness, number of cores, load ratio) or surface treatment, even in the case of labile and sensitive products. This very innovative multipurpose technology can be declared available for the mass production of microparticles.
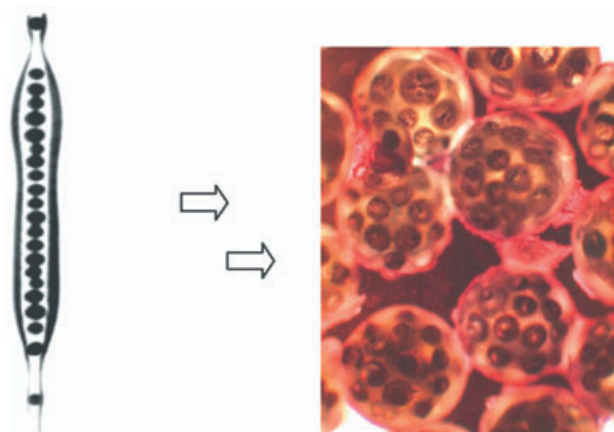

Figure 5. In-flight photograph of the natural breakup of a coaxial jet of blue ink surrounded by a photopolymer (SK9); multi-core microcapsules produced after UV curing (optical microscopy image).

range, more readily than other competing mechanic technologies, and yields remarkable size accuracy with negligible size dispersion and allowing surface and morphology

[1] a) X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, S. Weiss, *Science* **2005**, *307*, 538–544; b) S. Gouin, *Trends Food Sci. Technol.* **2004**, *15*, 330–347; c) H. Uludag, P. de Vos, P. A. Tresco, *Adv. Drug Delivery Rev.* **2000**, *42*, 29–64; d) D. T. O'Hagan, H. Singh, R. K. Cripta, *Adv. Drug Delivery Rev.* **1998**, *32*, 225–246.
[2] A. M. Gañán-Calvo, *Phys. Rev. Lett.* **1998**, *80*, 285–288.
[3] a) A. M. Gañán-Calvo, J. M. Gordillo, *Phys. Rev. Lett.* **2001**, *87*, 274501; b) A. M. Gañán-Calvo, J. M. Fernández, A. Marquez Oliver, M. Márquez, *Appl. Phys. Lett.* **2004**, *84*, 4989–4991.
[4] L. Rayleigh, *Proc. R. Soc. London Ser. A* **1879** *10*, 4–13.
[5] A. M. Gañán-Calvo, A. Barrero, *J. Aerosol Sci.* **1999**, *30*, 117–125.
[6] S. Xu, Z. Nie, M. Seo, P. Lewis, E. Kumacheva, H. A. Stone, P. Garstecki, D. B. Weibel, I. Gitlin, G. M. Whitesides, *Angew. Chem.* **2005**, *117*, 734–738; *Angew. Chem. Int. Ed.* **2005**, *44*, 724–728.
[7] S. Freiberg, X. X. Zhu, *Int. J. Pharm.* **2004**, *282*, 1–18.
[8] M. J. Blanco-Prieto, C. Lecaroz, M. J. Renedo, J. Kunkova, C. Gamazo, *Int. J. Pharm.* **2002**, *242*, 203–206.
[9] K. L. Kellar, M. A. Iannone, *Exp. Hematol.* **2002**, *30*, 1227–1237.

[10] a) M. Y. Han, X. Gao, J. Z. Su, S. Nie, *Nat. Biotechnol.* **2001**, *19*, 631–635; b) D. Wang, A. Rogach, F. Caruso, *Nano Lett.* **2002**, *2*, 857–861; c) V. Stsiapura, A. Sukhanova, M. Artemyev, M. Pluot, J. H. M. Cohen, A. V. Baranow, V. Oleinikow, I. Nabiev, *Anal. Biochem.* **2004**, *334*, 257–265; d) S. Sosnowski, J. R. Feng, M. A. Winnik, *J. Polym. Sci. Polym. Chem.* **1994**, *32*, 1497–1505; e) M. Bradley, M. Ashokkumar, F. Grieser, *J. Am. Chem. Soc.* **2003**, *125*, 525–529; f) W. Yang, D. Trau, R. Renneberg, N. T. Yu, F. Caruso, *J. Colloid Interface Sci.* **2001**, *234*, 356–362.

[11] a) A. Chauhan, C. Maldarelli, D. T. Papageorgiou, D. S. Rumschitzki, *J. Fluid Mech.* **2000**, *420*, 1–25; b) J. Meseguer, A. Sanz, *J. Fluid Mech.* **1985**, *153*, 83.

[12] A. M. Gañán-Calvo, J. M. López-Herrera, PCT/ES2003/000065.

www.small-journal.com