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FLOW FOCUSING® MICROENCAPSULATION: MUCH MORE THAN DRIPPING

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INTRODUCTION

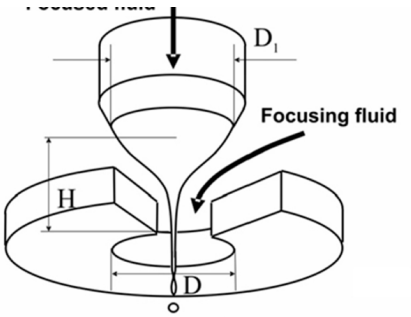
Numerous strategies and processes have been developed to obtain microparticles; the determinant step being the drop formation, which fixes the size distribution of the resulting microparticles. Depending on the physical properties of the fluids, different techniques or mechanisms are used to produce monodisperse drops. One of the more straightforward strategy is the formation of a single drop at a time, as in dripping processes [1-4]. Notwithstanding the very uniform microparticles obtained by this technique, it has some disadvantages as the drop-production rate is very low and the drop diameter scales with the diameter of the capillary or the pore, which makes it difficult to produce microparticles of a few micrometers or less.

The second strategy is the formation of numerous drops at a time, as in mixing or stirring processes[5], with scarce size predictability and homogeneity, or jet-disintegration techniques, in which a wide range of drop sizes is obtained, with different distributions depending on the Reynolds and Weber numbers of the jet. In particular, in laminar-jet disintegration or Rayleigh breakup [6] , the jet breaks up into uniform droplets due to capillary instability. One of the main features is that capillary jetting from a fluid source gives rise to droplets significantly smaller than dripping under the same operating conditions.

FLOW FOCUSING®

Flow Focusing® (FF) is a laminar-jet disintegration technology that uses the combination of a specific axisymmetric geometry and hydrodynamic forces to generate, under certain conditions, a microjet much smaller than the nozzle orifice, making the drop size independent of any geometrical dimension of the device: see Figure 1. One advantage of FF is the precise control of the microjet diameter that is achieved, and consequently of the droplet diameter, when the ratio of the jet inertia forces over the surface tension forces (i.e., the Weber number) is below a certain limit.

Figure 1. A schematic of the experimental flow-focusing setup. The disperse fluid is injected, with a syringe pump, through a capillary tube inside a chamber and pressurized by a continuous fluid supply. As the exit orifice of the chamber is facing the tip of the feeding tube, the focusing fluid stream forces the injected liquid (focused fluid) to exit the chamber through the orifice, producing a microjet much smaller than the exit orifice. The microjet proceeds downstream until it breaks up into a chain of nearly uniform drops.



Flow Focusing® was discovered in 1994 by Professor Alfonso Gañán-Calvo and developed by his research team. Since then, this micorfluidic technology has become the main business line of Ingeniatrics (Seville, Spain).
Based on this Proprietary Technology, Ingeniatrics has developed different tailor-made encapsulation processes of active substances, in order to stabilize them and provide protection from their external environment, as well as for controlled-release dosage, combination of mutually incompatible substances, etc.

Flow Focusing® is able to very gently produce and process aerosols and emulsions which, after the solidification process best suited to each system, gives rise to microparticles of the required composition and dimension, all the same as each other, something difficult to achieve by other technologies without further treatment.
In particular, here we show successful examples of the Flow Focusing® technology for the microencapsulation of different types of cells in monodisperse hydrogel microspheres using a Cellena® Flow Focusing® microencapsulator (Figure 2).

Figure 2. Cellena® User-friendly portable lab equipment for homogeneous encapsulation of living cells and microorganisms with sterile disposable nozzles.

CELL ENCAPSULATION BY CELLENA®

Cell encapsulation is an increasingly prominent and innovative technology that can provide solutions to complex problems within the area of biotechnology. In general, cell microencapsulation provides varying degrees of protection and isolation from their environment. It uses porous materials for the outer capsule which allow not only the diffusion of nutrients from outside to inside so that particles receive the necessary supplies for cell viability, but also permits the elimination/release of secretory products, both toxic and/or active substances, from cell metabolism.

Cellena® is the first bioencapsulation equipment developed by Ingeniatrics that is based on the Flow Focusing® technology. Its design allows encapsulation of cells / organisms in sterile conditions in the particle size required depending on the application type. Main features are:

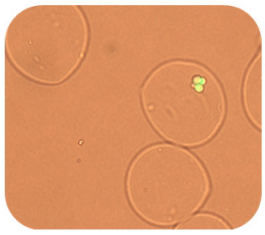
- Methodology in sterile conditions.
- Choice of particle size depending on the application, i.e. 90µm, 120 µm, 200 µm, 300 µm ... up to 500 µm
- Individual encapsulation of microorganisms in very small particle sizes.
- Uniformity of particle size, with reproducible composition.

- Very gentle technology that does not compromise cell viability.
- Validated for polymers solidified by ionic gelation: alginate, cellulose, etc

By means of Cellena® it is possible to produce monodisperse alginate microparticles containing individual bacteria, yeast and human stem cells (Figure 4). Alginate particle sizes were reproducibly selected from less than 100 µm to over 600 µm depending on the final application. Some of the main applications are:

- Cell susceptibility studies and microbial analyses
- Identification of potential biodiversity libraries
- Discovery of new, strong activity molecules
- Cell therapy
- Logistics
- Culture alternative for non-cultivable cells and organisms

One of the most important specific advantages of Cellena® is its unique ability to non-aggressively generate particles/micro-reactors of uniform and very small size that can be employed to address certain areas of microbiological research, in which it is essential to investigate microorganisms individually, without interference from other cells or strians present in the sample and speedily assessing the influence of various external factors (temperature, additives in the matrix like salts, antibiotics, cytotoxins, antibodies, enzymes, etc.) during a short number of division cycles. Some applications of individual cell-microencapsulations are:



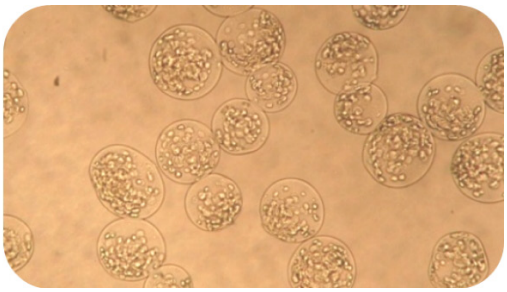


Figure 4. Left: Individual cell microencapsulation (fluorescent); Right: microencapsulated cells after growth.

- Evaluate the effect of a substance individually.
- Permit the individual growth of microorganisms while interacting with each other and with their external environment through extracellular signals.
- Discriminate rapidily the proliferation capacity of each cell within a population.
- Provide a highly sensitive detection method that can easily distinguish microcolonies of 30-100 individuals by flow cytometry.
- Reduce test times, speeding up and increasing the number of tests.

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Maria Flores is an Organic Chemistry (with a degree from UAH) and a PhD from Universidad Autónoma de Madrid (Spain). Since her PhD on inositolphosphogycans as insulin second messengers, she has been working in Medicinal Chemistry including Chemical Synthesis, Structure Activity Relationships and Molecules of Biological Interest. She joined PharmaMar in 2001 to continue working with Active Molecules, marine compounds with cytostatic activity. In 2004 she moved to Seville and joined Ingeniatrics becoming the Director of Research and Development. Ingeniatrics' R&D Department is focused on the development of microencapsulation applications of own proprietary technologies Flow Focusing® and Flow Blurring®. Main sectors of interest are Pharma, Food and Biotech industries.

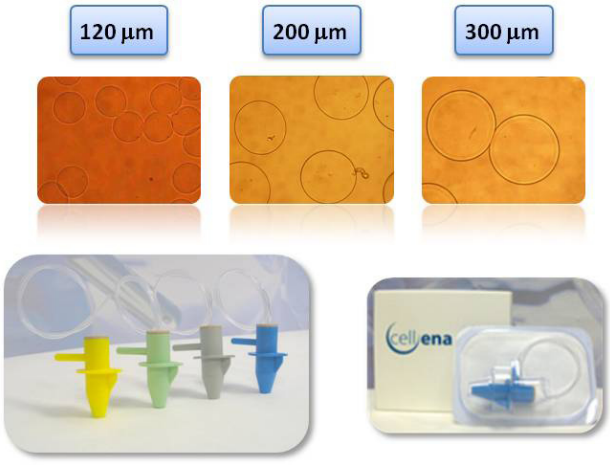

Figure 3. TOP: Examples of alginate microparticles obtained sterile disposable nozzles. BOTTOM: Disposable nozzles packed into sterile blisters designed for a fast and comfortable use preventing contamination.