The physics of pressure powered micro-flow focusing device for the encapsulation of live cells

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ABSTRACT

Micro flow focusing devices (mFFD) have emerged as a powerful tool for cell encapsulation and the formation of micro-emulsions. In the frame of live cell encapsulation, a micro-FFD using alginate solutions for the capsule and powered by highly controllable pressure pumps has been developed. This work deals with the different aspects of the problem: the rheology of alginate solutions, the flow regimes and flow charts, the influence of the different parameters including a scaling analysis and considerations on the thread break-up. Experimental observations of capsules produced in the microsystem are finally shown.

Keywords: encapsulation, alginate, flow-focusing device, flow regime, flow chart.

INTRODUCTION

Encapsulation of live cells is essential for many medical and biological applications. Encapsulated cells in gelled microporous matrix are protected from aggressions from the surrounding environment but, allowing to the size of the pores, they can exchange nutrients and ions through the porous shell. Hence cells can stay viable and functional inside their capsules.

Micro FFDs are presently the best solution to produce monodispersed capsules [1-3]. A schematic of a typical mFFD is shown in figure 1. The encapsulant is a liquid polymer diluted in an aqueous solution, flowing in the central channel and transporting the cells, which is later gelled to form a solid capsule. Alginate solutions are often used as encapsulant because of their biocompatibility and commercial availability [4,5]. The continuous phase is usually a mineral oil, without surfactants for biocompatibility constraints.

In this work we present the physical behavior of a mFFD powered by highly controllable pressure pumps (Fluigent ®) [5,6]. The rheological behavior of alginate solutions is investigated first, and then the different flow regimes related to the inlet pressures are determined. Flow charts for the three parameters of interest, i.e. the output frequency, volume and spacing of droplets are drawn and an analytical model predicting the behavior of the system is proposed in the case of sufficiently small liquid velocities. Considerations on the thread breakup are given next; it is shown that thread elongation is connected to the rheology of alginates. Finally experimental results of cell encapsulation are shown.

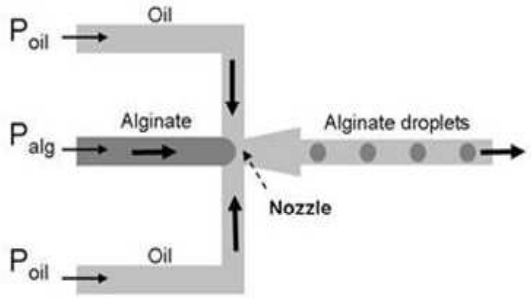

Figure 1: Sketch of a flow focusing device for the production of aqueous droplets in an organic continuous phase.

1 ALGINATE RHEOLOGY

Semi-concentrated alginate solutions (1 to 2 wt %) are very viscous fluids which show a clear visco-elastic, non-Newtonian behavior. Viscosities of such solutions depend on concentration, temperature and shear rate. Especially, it is shown that alginate shear viscosity follows a Carreau-Yasuda law (fig.2)

$$\eta(\dot{\gamma}) = \eta_0 \left[1 + (\tau\,\dot{\gamma})^{a}\right]^{\frac{1-m}{a}} \qquad (1)$$

where η_0 is the viscosity at zero shear rate, $\dot{\gamma}$ is the shear rate, τ the relaxation time, $a = 1.2$, and m is a constant that depends on the viscosity. Relation (1) shows the shear-thinning behavior of alginate solutions [5,7]. Most of the time, alginate viscosity is quite high, about 100 to 2000 times that of water, depending on the concentration and shear rate.

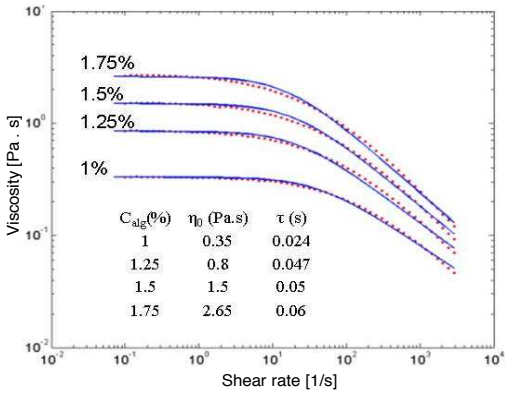

Figure 2: Alginate viscosity vs. shear rate: Fit of the Carreau-Yasuda law on the data for 1, 1.25, 1.5, 1.75wt% alginate concentration. Dynamic viscosity measurements are carried out in a Paar-Physica MCR300 rheometer using a cone plane geometry with a cone angle of 1° and a diameter of 50mm.

2 FLOW REGIMES

Different flow regimes can be observed in a FFD owing to the inlet pressures [8]. In a diagram (P_i,P_e), where *i* and *e* respectively denote the dispersed (alginate) and continuous (organic) phases, a map of the flow regimes has been determined (fig.3). It is found that the flow regimes are delimited by straight lines corresponding to a fixed value of $p^*=P_i/P_e$. For our application, the sector (2) corresponding to the droplet regime is recommended. For high inlet pressures, a special regime called the jetting regime is observed, whereas at low pressures, a dripping regime is observed [9]. In order to obtain reproducible, well calibrated capsules, the dripping droplet regime is recommended.

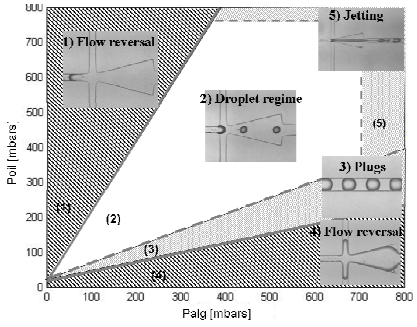

Figure 3: Flow regimes in a pressure powered flow focusing device.

3 FLOW CHARTS

In the perspective of capsule production, three parameters are of great importance: droplet emission frequency, droplet volume and droplet spacing [8]. The aim is to produce capsules of a given volume at a sufficiently high frequency with a sufficient spacing. Experimental determination of the values of these three parameters has been carried out; it is shown in figures 4 and 5 in a (P_i,P_e) diagram.

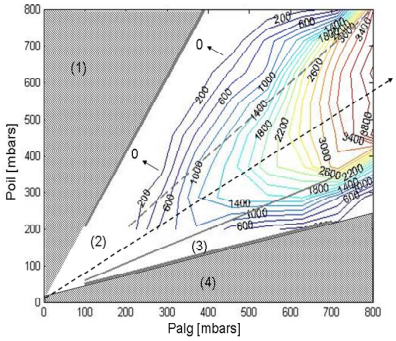

Figure 4: Contour plot of the droplet emission frequency, showing a ridge of maximum value [8].

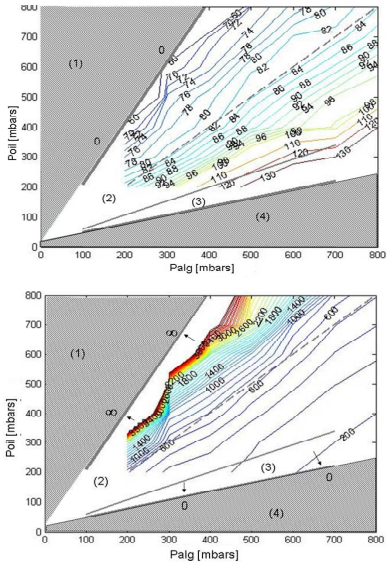

Figure 5: Contour plot of the droplet apparent diameter and spacing [8].

A locus of maximum output frequencies is determined corresponding to a fixed value p_f^*. For a large p^*, frequency increases when increasing P_i. Above the threshold p_f^*, the droplets are becoming large and their friction (function *f*) suddenly increases, reducing the

emission frequency. On the other hand, droplet spacing and size are almost constant along each line P_i/P_e=cste.

4 ANALYTICAL MODEL AND SCALING LAWS

An analytical model based on the flow resistance of the different branches of the device has been produced, assuming low velocities and averaging the fluctuations due to the formation of a droplet [5].

Using the notations of figure 6, the pressure drops in each branch can be written under the form

$$\begin{aligned} P_e - P_+ &= R_e Q_e \\ P_i - P_+ &= R_i Q_i \\ P_+ &= R_s (2Q_e + Q_i) f(Q_i/Q_e) \end{aligned} \quad (2)$$

where f is a function of the ratio Q_i/Q_e which takes into account the increase of pressure drop due to the droplet flow in the outlet channel. For the droplet regime where droplets have a size approximately equal to that of the cross section of the channel and are separated by relatively large distances, the function f can be considered constant and close to 1.05-1.1.

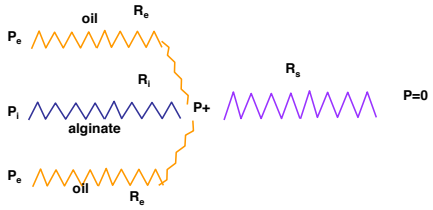

Figure 6: Sketch of the hydraulic resistances of the different branches of the FFD. The indices *i, e, s*, respectively stands for internal (alginate or water) phase, external (oil) phase, and outlet.

By resolving this system (2), we can derive a general relation between the flow rate ratio $q^* = Q_i/Q_e$ and the driving pressure ratio $p^* = P_i/P_e$ which can be simplified by taking into account that the flow rate of the alginate phase is much smaller than that of oil phase [5]

$$q^* = C(p^* + D - 1) \quad (3)$$

with $C = R_e + 2R_s f/R_i$, $D = R_e/R_e + 2R_s f$. The hydraulic resistances R_e, R_i, R_s are determined using the Washburn law [10] adapted for rectangular channels [11]. The hydraulic resistance R_i makes use of the expression of the viscosity of alginate solution given in section 1. This model has been checked against experiments and can be used to predict the influence of different parameters such as the fluid viscosity [8]. It has also been used to define scaling laws. It is shown that homothetic devices (with a scale ratio λ) behave similarly if the inlet pressures are in the ratio $1/\lambda$

$$P_{alg}^{(2)} = P_{alg}^{(1)}/\lambda \text{ and } P_{oil}^{(2)} = P_{oil}^{(1)}/\lambda \quad (4)$$

The emission frequencies f_r are then in the same ratio $1/\lambda$ and the droplet size (diameter ϕ) and spacing in the ratio λ

$$\begin{aligned} f_r^{(2)} &= f_r^{(1)}/\lambda \\ \phi^{(2)} &= \lambda \phi^{(1)} \end{aligned} \quad (5)$$

5 THREAD ELONGATION AND DROPLET FORMATION

A thread forms before a droplet detaches. It has been observed that the length of the thread before breakup is related to the elongational properties (elasticity) of the dispersed phase. Viscoelastic alginate solutions show a longer thread than regular Newtonian fluids—like water—and highly concentrated alginate solutions have longer thread than lightly concentrated solutions. As a matter of fact, we have observed a characteristic relation between the length of the thread just before the rupture (L_c) and its radius (R_c) (figure 7)

$$L_c/R_c = g(c) \quad (6)$$

where c is the alginate concentration and g an increasing function.

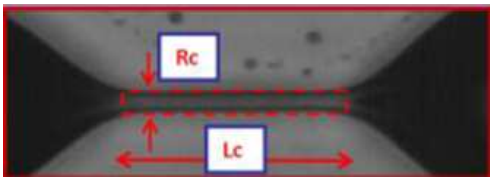

Figure 7: Thread elongating between the alginate feeding channel and the forming droplet.

By measuring the velocities of the two phases, the magnitude of the shear stress on the liquid filament has been deduced. It appears that the surface tension forces are much larger than the shear stress, i.e. the Capillary number

$$Ca = \frac{\eta \dot{\gamma} R_{thread}}{\gamma} \quad (7)$$

is small. It has been found that Ca < 0.1 in all our experiments. In the case of sufficiently long threads, satellite droplets appear at breakup. A model using the

numerical software Evolver can then predict the formation of such satellite droplets [12] (fig.8).

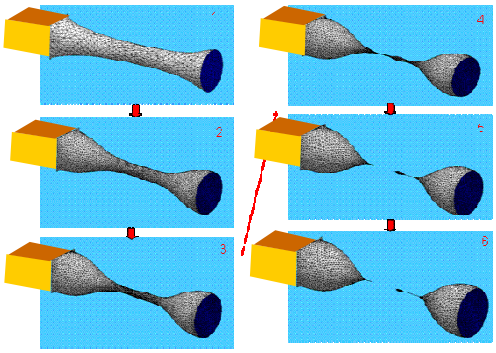

Figure 8: Elongation and thinning of a liquid thread with the formation of a satellite droplet.

6 RESULTS AND PERSPECTIVES

The understanding of the physical behavior of mFFD has guided the development and design of microsystems for the encapsulation of cells (fig.9). At the present time, the mechanisms of droplet formation are sufficiently well known to be able to produce a continuous flow of monodispersed droplets containing the cells. The data collected here have been obtained using Keltone HV solutions, but the quality of the alginate solution being of utmost importance for cell viability, purified alginate solutions will be used in the future developments.

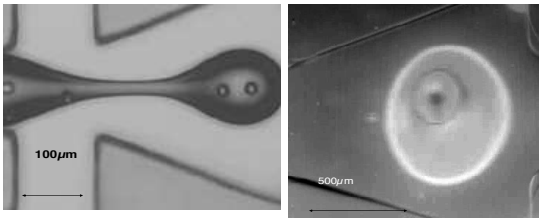

Figure 9: Jurkat cells trapped inside a droplet.

However, in the light of encapsulation, other steps have to be taken: the first one being the on-line gelling process required to obtain stable, gelled capsules encapsulating live cells. This step is not straightforward: it appears that the gelling products must not be in contact for too long with the cells for viability concerns. A solution similar to that of [13] is presently developed. The second difficulty is to only produce capsules containing cells. If no precaution is taken, a large number of capsules will not contain any cells. Gelled alginate capsules with no cells in it are not welcome in medical applications. This point will be presented in a following publication.

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