Online fabrication and characterization of capsule populations with a flow-focusing microfluidic system

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Abstract We have designed a microfluidic system that combines a double flow-focusing setup for calibrated capsule fabrication with a microchannel for the characterization of their mechanical properties. The double flow-focusing system consists of a first Y junction to create the microdroplets and of a second Y junction to introduce the cross-linking agent allowing the membrane formation. The human serum albumin (HSA) aqueous solution for the dispersed solution, hydrophobic phase for the continuous solution and cross-linking agent solution are introduced by means of syringe pumps. A wavy channel after the second junction allows to control the reticulation time. A cylindrical microchannel then enables to deform and characterize the capsules formed. The mechanical properties of the capsule membrane are obtained by inverse analysis (Chu et al. 2011). The results show that the drop size increases with the flow rate ratio between the central and lateral channels and does not change much regardless of the flow rate of the reticulation phase. The mean shear modulus of the capsules fabricated after 23 s of reticulation is of the order of the surface tension of HSA solution with Dragoxat indicating that the reticulation time is too short to form an elastic membrane around the droplet. When the reticulation time is increased to 60 s, the membrane shear modulus is multiplied by a factor of 3 confirming that a solid membrane has formed around the drop.

Keywords: Flow-focusing Microfluidic System, Capsule Fabrication, Capsule Characterization, Two-phase Flow

1. Introduction

A capsule consists of a liquid droplet enclosed by a thin deformable membrane. Capsule applications are found in many domains such as in the pharmaceutical (Cole et al. 2008), cosmetic (Miyazawa et al. 2000) and food industries (Gibbs et al. 1999). Encapsulation allows to protect the internal substance and to control its release via the control of the membrane properties.

One conventional two-step process used to fabricate capsules is based on the generation of droplets by emulsification, followed by the encapsulation of the droplets with a solid membrane. The membrane may be created by reticulation of proteins (Edwards-Lévy et al. 1993, Hurteaux et al. 2005), by spray-coating a film-forming material around the microdrops (Guignon et al. 2002) or by precipitation of polymer on the droplet surface (Ribeiro et al. 1997). Another type of fabrication process is the technique based on spray-drying method (Li et al. 2008): microdroplets are atomized through a nozzle by a pneumatic atomization or a rotary nozzle and dried by a filtered and heated air flow to form dried capsules. These different conventional techniques are largely used in industrial applications because they allow the production a large quantity of capsules. However they do not allow the fabrication of monodispersed capsules.

In order to improve the homogeneity in capsule size, microfluidic techniques have

been proposed. Microfluidic devices allow the fabrication of calibrated drops by injecting a disperse phase into a flowing immiscible continuous phase. Monodispersed microdrops can be produced using a T-junction microchip (Garstecki et al. 2006), a flow-focusing microchip (Yobas et al. 2006) or a microfluidic system based on co-axial cylindrical channels (Liu et al. 2009). The encapsulation process can be performed outside (outline) or inside (online) the microfluidic system. For outline encapsulation, monodisperse drops formed at a cross-junction are collected in a reservoir containing a cross-linking solution (Huang et al. 2007; Yeh et al. 2009). The mechanical properties of the capsule membranes tend to be not uniform because the drops do not reach the reservoir at the same time. For online capsule fabrication, drops that are formed at the first cross-junction are reticulated at a second cross-junction, through which a reticulation phase is injected. A downstream channel allows the control of the polymerization time of the capsules before collection (Zhang et al. 2006). The fabricated capsules then have a controlled membrane thickness and a controlled size.

Another issue is the determination of the membrane mechanical properties needed to optimize capsule fabrication. Different methods have been used to measure the membrane mechanical properties of a single capsule. Compression experiments consisting in compressing a capsule between two parallel plates and measuring the compression force as a function of the plate distance are used to characterize millimetric capsules (Carin et al. 2003). For micrometric capsules, micropipette aspiration experiments measuring the capsule length aspirated into the micropipette under various pressures are used (Needham and Zhelev. 1996). Fery and Weinkamer (2007) used an atomic force microscope (AFM) to deform the microcapsule under a known force. Both techniques are difficult to implement due to the small capsule size. Entire microcapsule populations have been successfully characterized with an inverse analysis technique based on microfluidic experiments (Lefebvre et al. 2008; Chu et al. 2011). However, no technique exists to fabricate and characterize microcapsules at the same time.

We have designed a microfluidic system that combines a double flow-focusing setup for drop generation and encapsulation by reticulation with a microchannel for the characterization of the capsule mechanical properties. The objective is to optimize the system to fabricate calibrated capsules and measure the geometric and mechanical properties of the capsule formed. In this paper, we study the formation of drop of albumin aqueous solution in Dragoxat, a hydrophobic liquid that offers many applications in cosmetic and pharmaceutical domains (Hurteaux et al. 2005). A cross-linking agent, i.e. terephtalyol chloride is used to form a network of cross-linked protein around the albumin droplet.

In section 2, we present the design and technique of fabrication of the microsystem. The fluid preparation and manipulation are also described in this section. The results of droplet and capsule fabrications are presented in section 3 before concluding in section 4.

2. Materials and methods

2.1 Microsystem design and fabrication

In order to fabricate calibrated droplets and therefore capsules, we have designed a flow-focusing microchip as illustrated in Figure 1. The important geometric parameters for droplet fabrication are: the width W_1 and the depth h_1 of the central channel (channel 1), the width W_2 of the lateral channels (channel 2), and the intersection angle α between the lateral and central channels. The droplets are formed at the first junction between channels 1 and 2. A reticulation phase is injected into channel 3 to enable the formation of the membrane around the droplets when they pass the second junction between channels 1 and 3. The length L_4 of the wavy channel (channel 4) allows the control of the reticulation time. Its waviness favors mixing and therefore an uniform concentration in reticulation phase in the

channel. The width W_4 and depth h_4 of channel 4 are larger than for channel 1 to slow the capsule down (Table 1). For capsule characterization, a cylindrical channel of internal radius R is inserted inside channel 5. The capsules are collected in reservoir 6.

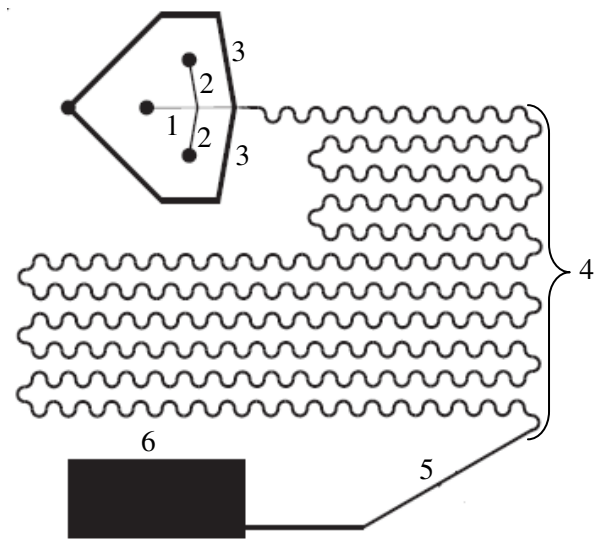

Figure 1: Microfluidic system for fabrication and characterization of microcapsules.

The mould master is manufactured by photolithography. Briefly, SU8 photoresist is deposited on a silicon wafer by spin coating. The photoresist is then exposed to ultraviolet light through a photomask to create a latent image of the circuit. The system is then immersed and agitated in a developer to dissolve the unexposed parts. We then obtain the mould master consisting of a SU8 photoresist patterned on a Silicon wafer.

The procedure of fabrication of the Polydimethylsiloxane (PDMS) microsystem is described by He (2009). A mixture of PDMS with curing agent (9:1 mass ratio) is poured onto the mold master. The system is then degassed to remove the air bubbles and heated in the oven at 70°C for 2 hours. After the PDMS mould is peeled off the master, five holes are drilled into it for the injection of the solutions and collection of the capsules. The PDMS is then bonded onto a glass substrate using plasma treatment to close the channels. The system is only used after a few days for the PDMS to retrieve its hydrophobicity.

In this paper, 4 chips have been studied. The geometric parameters are indicated in Table 1. Chips 1 and 2 are used to study the fabrication of droplets. Only chips 3 and 4 are used to fabricate capsules.

Chip	W_1	W_2	h_1	W_4	h_4	L_4 (cm)
1	103	215	50			
2	103	215	115			
3	103	215	115	300	200	67,5
4	109	110	88	290	320	100

Table 1: Chip geometric parameters. Units are in µm except for L_4 which is in cm.

2.2 Fluid phases

The disperse phase consists of a 20% human serum albumin (HSA) solution in a phosphate buffer pH 9.8 with viscosity μ = 3.31 mPa.s at 25°C. The continuous phase consists of a biocompatible fatty ester Dragoxat (2-ethylhexyl 2-ethylhexanoate, Symrise) with viscosity μ = 3.5 mPa.s at 25°C. The surface tension between Dragoxat and HSA solution is γ = 0.006 N/m (Tensiometer Kruss DSA10 Mk2). The reticulation phase containing 2.5% w/v of terephthaloyl chloride (TC) in Dragoxat is obtained by mixing 0.25 mg of TC powder in 10 ml of Dragoxat and stirring for 2 h with a magnetic stirrer. The TC particles remaining in the solution after mixing are eliminated using a filter of size ~ 1 µm to avoid clogging of the microfluidic channels.

2.3 Experimental procedure

For the droplet fabrication, channel 3 is closed. Albumin solution is injected into channel 1 at a flow rate Q_1 using a syringe pump (KD Scientific). The Dragoxat is injected into lateral channels 2 using a two-channel syringe pump producing equal flow rates Q_2 in each branch. Droplets are formed at the first junction. Their motion is observed with a microscope (Optika) and recorded with a high-speed camera (IF 800, Japan) at 150 frames/s with a shutter speed of the order of 10^{-3} s. The microscope is focused on the middle plan of the channel to obtain the cross section of the drop in its axial plane. The drop size is characterized by measuring the droplet

length L_g in the central channel (Figure 2).

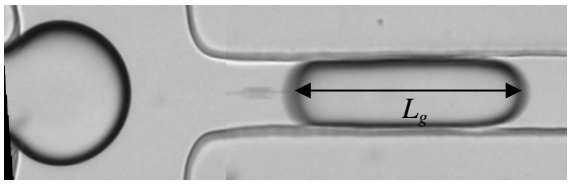

Figure 2: Droplet fabrication at the first junction.

Before the capsule fabrication, Dragoxat is injected into channel 3 to stabilize the flow. When the flow is steady, the reticulation phase is injected instead at a flow rate Q_3 to enable the drop reticulation. As the size of channel 4 is very large, the capsules assume a spherical shape from the second junction on and continue to polymerize in channel 4. The capsule size is characterized by measuring the droplet size in channel 1 because their volume only differs by a thin membrane. The capsule radius a is obtained by calculating the volume of the droplet assuming elliptic shape. The reticulation time is the time the capsule takes to travel along the wavy channel.

$$t_r = \frac{L_4}{v_g} = L_4 \times \frac{W_4 \times h_4}{Q_1 + 2 \times Q_2 + Q_3} \qquad (1)$$

In order to measure the elasticity of the capsule membrane, a cylindrical microchannel of internal radius R = 75 µm is inserted into channel 5 to deform the capsules. The capsule deformed shape is compared with predictions obtained numerically for a capsule with the same size ratio a/R, under the same flow conditions. The unknown shear modulus corresponds to the best fit (Lefebvre et al. 2008; Chu et al. 2011).

3 Results and discussions

3.1 Effect of the continuous phase on the drop size

We first close channel 3 to examine the variation of the drop size as a function of the flow rate ratio between the central and lateral channels. The results measured in chips 1 and 2 are shown in Figure 3. The drop length normalized with the channel dimensions increases linearly with the flow rate ratio Q_1/Q_2. The normalized droplet size measured for chips 1 and 2 falls approximately on the same linear regression curve as shown by He et al. (2009). The slug length is thus approximated:

$$\frac{L_g}{h_1} \approx 14.7 \frac{W_1}{W_2} \frac{Q_1}{Q_2} + 3.8 \frac{W_1}{W_2} \qquad (2)$$

The dispersion may come from the fuzziness of the droplet image. The droplet occasionally travels at a too high speed for a clear image to be taken (the camera has a limited exposure time). Depending on the velocity of the droplet, the error of the experimental measurement might reach 6%. Another source of error is related to the flow instability resulting from the pump setting.

The influence of the chip geometry is also indicated. We observe that in chip 1, droplets can be fabricated in a larger Q_1/Q_2 range (0.1 - 1.1) than in chip 2 (0.1 - 0.5). Because chip 2 being deeper than chip 1, the flow rate Q_2 of must be larger to form droplets.

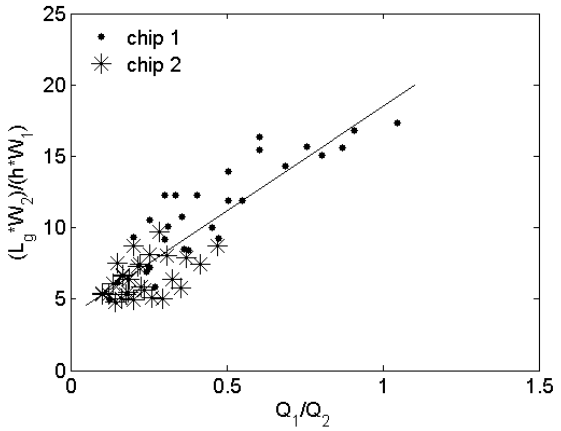

Figure 3: Variation of the normalized droplet length with the flow rate ratio Q_1/Q_2 in chips 1 and 2.

3.2 Effect of the reticulation phase on the drop size

We injected the reticulation phase through channel 3 to examine whether its flow could influence and perturb the drop formation occurring at the upstream bifurcation. It is of interest that the drop size that is also the capsule size does not change much regardless

of the flow rates of the reticulation phase (Figure 4). We conclude that the reticulation phase flow does not influence significantly the capsule size.

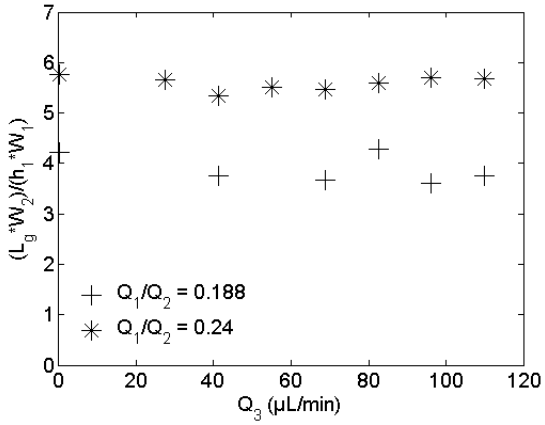

Figure 4: Variation of the normalized droplet size with the flow rate of the reticulation phase in chip 2.

3.3 Shear modulus measurement

The inverse method (Chu et al. 2011) is used to measure the capsule shear modulus. We first measure the shear modulus of the capsule population fabricated with chip 3. We fix Q_2 = 33 µL/min and vary Q_1 from 7 to 15 µL/min to change the capsule size. The flow rate of the reticulation phase is Q_3 = 25 µL/min. The reticulation time is about 23 s. An example of the capsule shape flowing down the cylindrical channel is shown in Figure 5a.

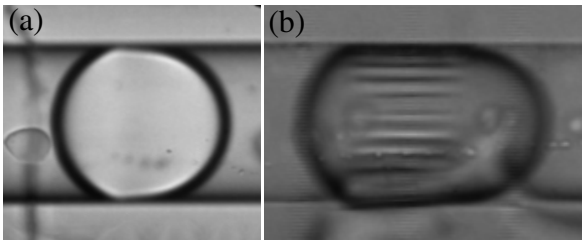

Figure 5: Example of fabricated capsules flowing down the cylindrical microchannel (a) t_r = 23 s, a/R = 1.08 and (b) t_r = 60 s, a/R = 1.17.

The shear modulus measured for capsules fabricated after 23 s are shown in Figure 6 as a function of the size ratio a/R. The mean value of the shear modulus is G_s = 0.004 N/m with a standard deviation of 0.001 N/m. This value is of the order of the surface tension between the Dragoxat and HSA (0.006 N/m). The present reticulation time of 23 s is therefore too short for a solid membrane to form around the droplet.

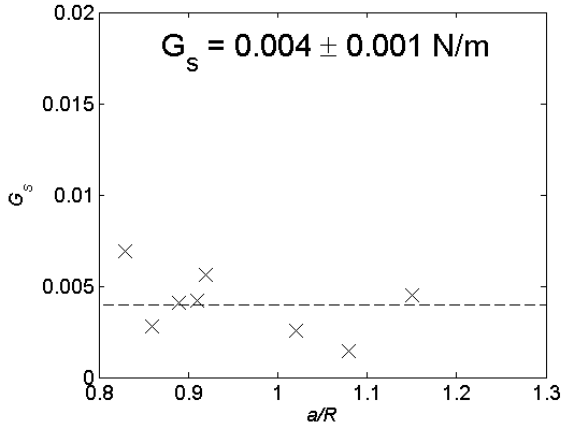

Figure 6: Membrane shear modulus of the capsule fabricated for a reticulation time of 23 s as a function of the capsule size ratio a/R.

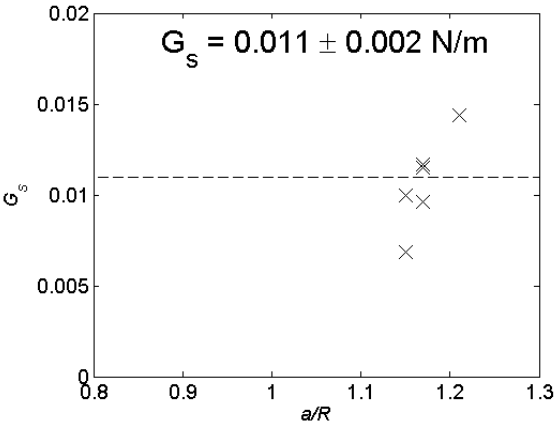

Figure 7: Membrane shear modulus of the capsule fabricated for a reticulation time of 60 s as a function of the capsule size ratio a/R.

In order to increase the reticulation time, chip 4 was made with a longer wavy channel. We also fix Q_2 = 25 µL/min, Q_3 = 33.8 µL/min and vary Q_1 from 6 to 9.2 µL/min to change the capsule size. The reticulation time is about 60 s. Deformed wrinkled capsules are observed in the cylindrical channel (Figure 5b). After 60 s of reticulation, the mean shear modulus of the capsule membrane is measured to be G_s = 0.011 N/m. This value is nearly the double of the surface tension between the Dragoxat and the HSA solution (0.006 N/m). It confirms that a membrane is formed around the drop.

4. Conclusions

We have designed and used a single microfluidic system to fabricate and directly characterize microcapsule populations. The microdroplet size depends only on the flow rate of the continuous and dispersed phases. It is independent of the flow rate of the reticulation phase. The mean shear modulus of the capsules fabricated after 23 s of reticulation is of the order of the surface tension of HSA: such a low reticulation time is too short to form an elastic membrane around the droplet. After 60 s of reticulation, a capsule is however formed. This result shows that the system is capable of fabricating and characterizing capsules at the same time.
The use of a microfluidic system offers many advantages. It allows the fabrication of calibrated capsules by forming droplets of a defined size, followed by a controlled reticulation. We can also measure the geometric and mechanical properties of the capsules in situ. A feedback control on the flow rates of the different phases according to the measured properties would enable to optimize the fabrication conditions on demand.

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