Monodisperse microcapsules with controlled interfacial properties generated in microfluidic T-shape junction

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Résumé :

Les microcapsules sont des gouttes enrobées par une membrane. Un dispositif de fabrication de micropcapsules monodisperses en chitosan a été mis au point. Les propriétés mécaniques de la membrane peuvent être finement contrôlées dans une large gamme en variant les concentrations des réactifs ainsi que le temps de polymérisation. La déformation de ces capsules dans un écoulement élongationnel est reproductible et en accord avec la théorie dans le régime des faibles déformations. Nous avons identifié une instabilité membranaire qui donne naissance à une modulation de la forme que l'on peut identifier à des plis de faible amplitude. La longueur d'onde des plis est proportionnelle à la concentration en surfactant, un paramètre clé de la synthèse de ces microcapsules.

Abstract :

Monodisperse chitosan microcapsules were prepared on the base of microfluidic T-shape junction. Via adjusting the concentrations of complexants and membrane polymerization time, we are able to tune the interfacial properties (like surface shear elastic modulus) in a wide range. The capsule deformation in elongation flow exhibited good agreement with the theory of small deformation. We identified a membrane instability that give rise to a modulation of the shape of the membranes, wrinkles of small amplitude. The wavelength is proportional to the concentration of surfactant, a key parameter of the synthesis of these microcapsules.

Mots clefs : microcapsule, propriétés interfaciales, instabilité membranaire

1 Introduction

Microcapsules are widely found in the nature (e.g. red blood cells and some bacteria), as well as in artificial products. Generally, they are considered as liquid, solid or gaseous cores [1] surrounded by the thin shells that have the abilities of protection the core ingredients [2] or controlling the release [3]. Artificial microcapsules are exhibiting great interests in various applications such as food additives or cosmetic products encapsulation and medical drug delivery. Indeed, to model the biological cells using artificial microcapsules is a good way to verify the scope of applications of various membrane constitutive laws [4, 5] for different capsule shell properties.

The challenging work could be the microcapsule encapsulation with well-define properties of the membrane, for example the controlling of capsule sizes and interfacial rheology properties [6, 7]. The interfacial properties of microcapsule mainly depend on the synthesis procedures and notably on the fact that the membrane grows from the chemical polymerization located on the interface of the initial droplet. Typical membrane of capsule should be biocompatible and biodegradable, more importantly has rather well properties of reversible deformability when subjected in the flows. Capsules made of nylon [8] presented large deviation of deformation for different capsules fabricated in the same lot in the elongation flow. Polysiloxane capsules [9] exhibited linear deformation in shear flow which is in good agreement with the theory, but membrane fracture may occur at the deformation between 8% and 10% that showed too weak resistance for imposing strain. In these strategies, it is hard to obtain microcapsules with significantly similar shell mechanical behaviors, and to tune the range of ability of response to the imposed stress. The previous experimental studies showed wrinkling instabilities happened for polysiloxane capsules which may correspond to the osmotic pressure, membrane pre-stress or bending elasticity [10]. Unfortunately, the wrinkles on such kind of capsules were too weak to analyze quantitatively (wavelength and wavenumber).

Taking advantage of oppositely charged polyelectrolytes/surfactants [11, 12], we are able to control the interfacial properties by varying the determining parameters. Chitosan capsules exhibit great expecting features to study since the carbonhydrate backbones of chitosan molecules (shown in figure 1) show substantially stiff characteristics that contributes to the elasticity of membrane. The preparation of monodisperse microcapsules involves emulsification of the disperse phase into the continuous phase which both are immiscible. Recently, the precise flow control afforded by microfluidic technique provides a good means to fabricate the homogeneous monodisperse capsules [7, 13] with controlling the membrane properties.

Here we propose a straightforward approach to generate monodisperse biopolymer microcapsules with controlling properties on the base of microfluidic T-shape chip. The droplets with designed size firstly produced in the T-shape chip were considered as the templates surrounded by the interfaces of water/oil. These templates, afterwards, were transferred into a reaction reservoir where the membrane was formed. By controlling the polymerization time and concentrations of polyelectrolyte and surfactant, we are able to tune the interfacial properties of capsules in a wide range. A cross-slot setup generating elongation flow [8] was used to detect the shell properties. Results showed that the deformation of different capsules produced with same controlling parameters was significantly similar. For small deformation ($D_\infty <$ 9%), the deformation increases linearly with the elongation stress, which is consistent with the theory prediction [14]. The membrane instabilities such as wrinkles and folds were observed, and apparent wrinkling on the equators of deformed capsules allowed us to analyze the wavelength.

2 Experiment section

2.1 Materials and solution

Chitosan from Sigma-Aldrich Corp., with medium molecular weight and 75-85% deacetylation (DA), was used for all experiments in this investigation. In the acid condition, chitosan molecules carry positive charge and the charge density is often determined from the deacetylation and pH value. Millipore water (resistivity > 18 MΩ cm) with pH = 3 was used as the medium to solve chitosan powder. In terms of suspension of chitosan capsules, a certain fraction of glycerol has to be added into the chitosan solution in order to balance the density of capsules and external phase.

The anionic surfactant used in this study to precipitate capsule membrane is ammonium phosphatidic fatty acid (referring to PFacidYN) from Palsgaard , shown in figure 1. The detailed ingredients fraction of lecithinYN has been discussed in the previous publication [11]. The anionic surfactant was dissolved in the rapeseed oil from Brassica rapa.

Mineral oil and cyclohexane from Sigma-Aldrich company were used to rinse microcapsules after a certain polymerization time. Silicone oil AP1000 (offered by IMCD group) acted as the external phase to suspend microcapsules in elongation flow.

2.2 Microcapsules preparation

As mentioned above, chitosan is a kind of cationic polyelectrolyte carrying positively charged groups that presents good solubility in water under acid condition (e.g., pH<4), allowing it to form the membrane when contacted with oppositely charged surfactant, like PFacidYN in a rapid way [11, 15], shown in figure 1. The first rather thin shell was formed at the interface water/oil when the chitosan liquid droplet was placed in the oil phase containing PFacidYN molecules due to electrostatic absorption.

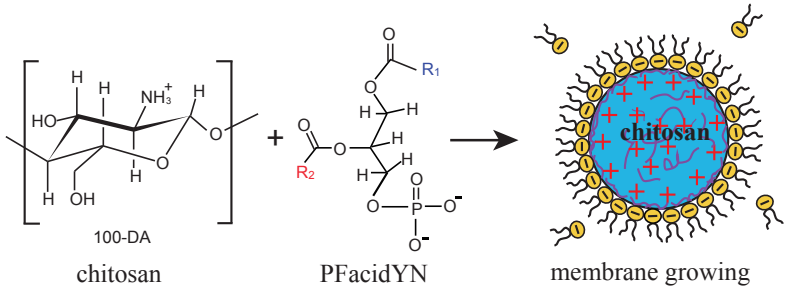

FIGURE 1 – Membrane complexation of chitosan and PFacidYN molecules

A new configuration of T-shaped microfluidic chip made of hydrophobic material resin was designed and manufactured by a 3-D printer (Formlabs Inc., Form 1+), to generate water-in-oil capsules. Unlike the traditional configures of T-shaped microfluidic chip [16], the intersection area in the direction of main flow (continuous phase) was narrowed in order to strengthen the shear forces breaking disperse phase jet flow, therefore, to improve the producing efficiency of capsules. This allows us to produce uniform size of capsule stably, particular for the high viscosity of dispersed phase. Figure 2 (a) shows the scheme of T-shape microfluidic chip, in which a round capillary with inner diameter 200 μm was place in the side branch that is perpendicular to the main channel. Continuous phase was injected from the entrance of main channel, whereas the disperse phase entered the chip through the capillary. The flow rates of both phases were precisely controlled by a controller system (Fluigent).

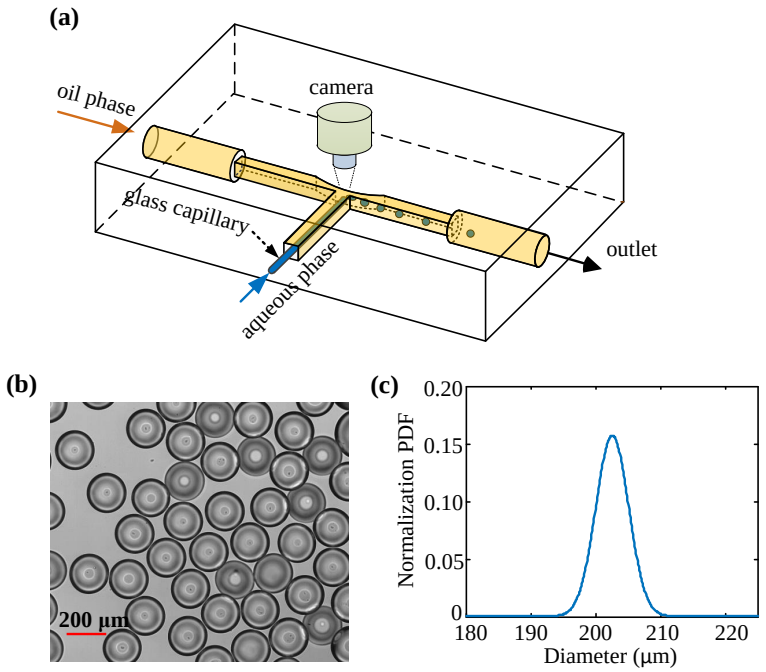

FIGURE 2 – (a) Schematic illustration of T-shape microfluidic chip used for the generation of emulsion drop templates. (b) Optical micrograph of chitosan capsules on substrate. (c) Size distribution of chitosan capsules.The experimental points were fitted with the probability density function.

To obtain the tunable interfacial properties, the procedure of capsule formation can be divided into three parts : aqueous core droplets formation, membrane growth and polymerization stop. Firstly, liquid droplets acting as templates were pre-formed in the T-shape chip where the size distribution can be adjusted via flow rate ratio of oil/aqueous phases. In this step, very low concentration of PFacidYN (< 0.02% w/w) was used in the oil phase in order to prevent coalescence happening. After sufficient number of capsules has been collected, the membrane formation of capsules was controlled by transferring liquid templates into a reaction reservoir which contained a designed concentration of PFacidYN. The residual time of capsule in the reaction reservoir varied from 2 to 25 minutes. When the desired time was reached, a large volume of mixture of mineral oil/cyclohexane (4 :1 v/v) was poured into the emulsification system to stop the membrane polymerization. And then, capsules were rinsed by cyclohexane twice. Figure 2 (b) and (c) shows the optical micrograph of capsules on the substrate and the size distribution, respectively. The size deviation of capsules used in this investigation is within 4%. After washing procedures, microcapsules were dispersed in silicone oil Wacker AP1000 to be streched in elongation flow. In this investigation, capsules used in all experiments have a mean diameter of 204 μm.

2.3 Optical flow cell

To build an elongation flow, a cross slot made of PMMA was fabricated, which consists in two perpendicular rectangular channels, as shown in figure 3 (left). In the cross area, the width and depth of channel is 1 mm. Microcapsules seeded in silicone oil AP1000 were pumped into the setup by a glass syringe mounted on a home-made syringe pump controlled by a PI actuator M235-52S. The outlets were connected to the ambient pressure, which allows to adjust the position of a capsule on the x-y plane and to keep it stop at the stagnation point. Capsule deformation was visualized by an inverted microscope Olympus IX-71 with a magnification of 10× or 20×. A high speed video camera (Photron Fastcam

SA3) enables us to acquire up to 5000 frames per second. Particle Tracking Velocimetry (PTV) [6] was employed to determine the rate-of-elongation in the cross slot. To minimize measurement deviation, we defined a region of interest (ROI) with a diameter of 600 μm around the stagnation point on x-y plane, distance within $\pm$100 μm from center-plane in z-direction, within which the elongation rate $\dot{\varepsilon}$ was considered homogeneous (standard deviation is below 5%). The image post-processing was performed with a Matlab code. Figure 3 (right) shows the capsule Taylor parameter, D, as a function of dimensionless time. Taylor parameter of capsule is defined as equation 1 :

$$D = \frac{L - S}{L + S} \tag{1}$$

where the L and S represent the major and minor axis of deformed capsule. When the deformation of capsule in ROI was saturated (shown in figure 3(b)), it was called infinity deformation denoted as D_∞. The detailed way to deform capsule in such cross-like channel can be seen in reference [6].

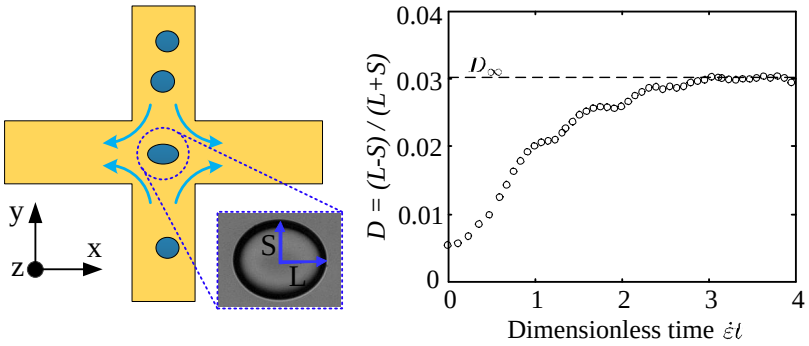

FIGURE 3 – Left : Elongation flow generated by a cross-slot. Right : Saturation of deformation variation with dimensionless time $\dot{\varepsilon}t$ for different elongation rates. In the ROI, all capsules can reach the steady-state deformation D_∞.

3 Results and discussion

3.1 Surface shear elastic modulus measurement

The detection of interfacial properties of capsules were mainly focused on the surface shear elastic modulus, G_s, which can be calculated from the asymptotic theory [17] for small deformation (D_∞ < 9%). G_s is inferred as equation 2 :

$$G_s = \frac{25}{6}\frac{\sigma R}{D_\infty} \tag{2}$$

where σ is the elongation stress imposed on the surface of capsule, which can be defined as $\sigma = \eta\dot{\varepsilon}$. R is the radius of studied capsule.

To measure the surface modulus G_s, firstly the steady-state deformation D_∞ for different capsules were captured with imposing various elongation stress. In figure 4, the steady-state deformation D_∞ varies with the stress which is in good agreement of the theory of small deformation. Secondly, we are able to determine the G_s value from the slope according to equation 2. We have verified that within small deformation capsules that were chosen to deform could recover to the initial shape completely just after stop the flow. The measurement standard deviation of G_s in this study is less than 6%.

TABLE 1 – Surface shear elastic modulus G_s variation with membrane complexation time. Capsule were made by 0.25% w/v chitosan, 0.36% w/w PFacidYN.

time t_r [min]	mean G_s [N/m]
2	0.111 ± 0.0058
6	0.230 ± 0.0193
12	0.422 ± 0.0371
25	1.023 ± 0.0490

As illustrated in figure 4, different microcapsules with the same formulation exhibit similar behavior, which means that capsules fabricated in our present method have same elastic properties of membrane. This is quite essential for some applications, for example suspension study.

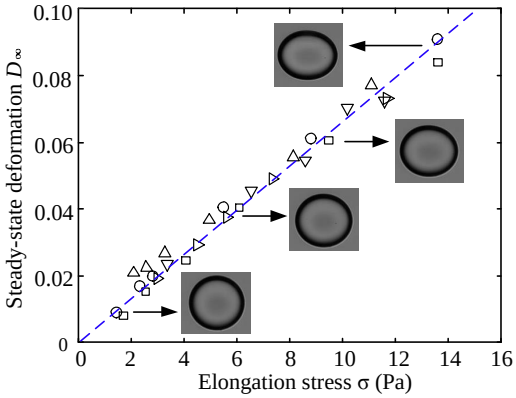

FIGURE 4 – The steady-state deformation as a function of elongation stress. Each type of symbol represents a capsule. Capsules were made by 0.11% w/v chitosan, 0.36% w/w PFacidYN, 2 min complexation time.

Table 1 presents the influence of complexation time on the surface shear elastic modulus of chitosan microcapsules. Four different retention time of capsule in reaction reservoir were studied with the constant concentration of chitosan (0.25% w/v) and PFacidYN (0.36% w/w). The mean G_s was calculated from 4∼6 capsules. Shear modulus increases almost linearly with the complexation time of membrane, varying from 0.11 N/m to 1.02 N/m, more than 9 times larger. As mentioned above, the process was dominated by the diffusion of PFacidYN molecules through the growing membrane. Unlike the characterization of capsule membrane properties in situ after fabrication in microchannels [18], the method of membrane polymerization used in this paper allows us to control complexation time until long time. This may be an efficient way to tune the interface properties of capsule membrane.

Apart from controlling the membrane complexation time, varying the concentrations of complexants is another way to adjust the shell interfacial properties. Figure 5 shows the G_s variation with the concentrations of PFacidYN and chitosan. Like the influence of complexation time, shear modulus can be modified linearly by varying the PFacidYN molecules concentration. A function G_s = 0.304 $C_{lec.}$ can be extracted from these points. Differently, when the concentration of chitosan is above a threshold value (0.5% w/v), a plateau appears where the G_s becomes independent on the concentration of chitosan. It means the above the critical concentration of chitosan, the limitating factor would be the mass of surfactant. Whereas, in the rising-up stage (figure 5 right), the membrane growth is limited by the quantity of chitosan molecules that encapsulated inside capsules.

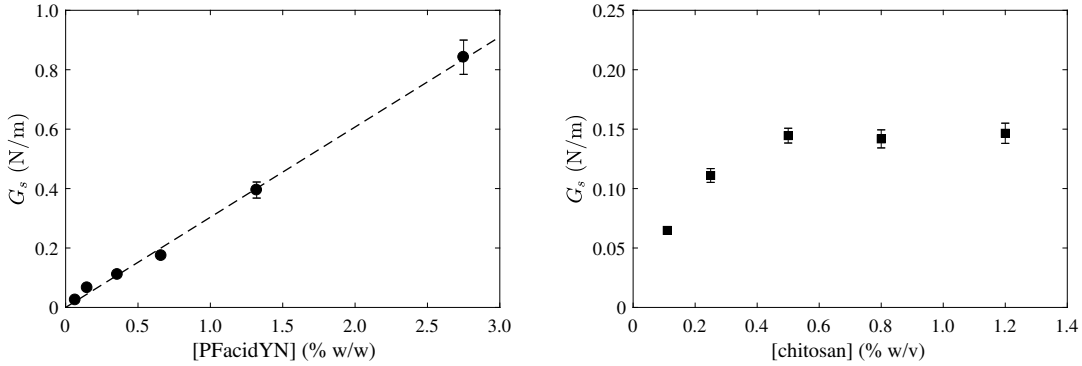

FIGURE 5 – Surface shear elastic modulus G_s as a function of the concentrations of complexants. Left : Effect of the concentration of PFacidYN, capsule with 0.25% w/v chitosan, 2 min complexation time. Right : Effect of chitosan, capsule with 0.36% w/w PFacidYN, 2 min complexation time.

3.2 Membrane instabilities : Wrinkles and folds

The membrane instability phenomenon was observed when the capsule was subjected in the elongation flow. As shown in figure 6, under low stress, the wrinkles is too weak to be detected using current observing method. With increasing the stress the wrinkles were appearing, however it seems that they merged into folds because it could simply a change of wavelength occurred while folds are deep and localized even if the periodic characteristic still exists. And the orientation of wrinkles were always parallel to the main axis of capsule. After the flow was stopped, all the capsules recovered to the initial situation, no wrinkles existing on the surface.

Since the capsule is a three-dimensional object, it is not easy to analyze the geometric characteristics of these wrinkles, like the amplitude and wave number. We consider the wrinkles in the center area of capsule surface (figure 6 square) are the typical shapes. Then it is possible to obtain the wavelength of wrinkle by processing the image. Figure 7 shows the linear relationship of wavelength of wrinkles and the concentration of surfactant. The higher concentration of surfactant, the larger wavelength happened. The massive surfactant contributes to thick shell leading to increasing membrane bending stiffness. Membrane with larger bending stiffness shows fewer wrinkles formation [19]. Note that in the current investigation, we are not able to observe wrinkle instability when concentration of PFacidYN is less than 0.66% w/w. Probably, they exist but very weak to detect due to the limiting of camera resolution when the shell thickness is small.

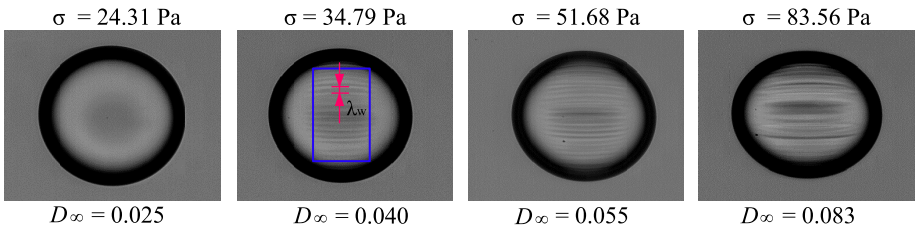

FIGURE 6 – Wrinkles and folds growing on the surface of chitosan capsules in elongation flow. Capsule fabricated by 0.25% w/v chitosan, 1.32% w/w PFacidYN, 2 min complexation reaction.

The presence of negative principle tensions [17] may dominate the process of wrinkling appearing. As the membrane of chitosan capsule is permeable for water molecules, some water molecules probably

could diffuse from the aqueous core into external oil phase by the presence of surfactant during the membrane growing, even the water solubility in oil is very low. However, this process may occur in nano-scale view because we didn't observe the apparent decreasing of capsule diameter after emulsification procedures. The osmotic pressure could be a factor that induces the negative tensions on the membrane. Wrinkles phenomenon was observed in polysiloxane capsule [9] made by oil-in-water emulsification even the membrane was under pre-stress. It means the osmotic pressure in this condition should be vanished. Therefore, it is hard to say if the osmotic pressure affects the wrinkling. There is another conceivable explanation that the wrinkling instability is dependent on the bending elasticity of membrane [10]. If we are able to measure the membrane bending stiffness in other ways, maybe it can provide an interesting technique to verify the wrinkles formation on the capsule surface.

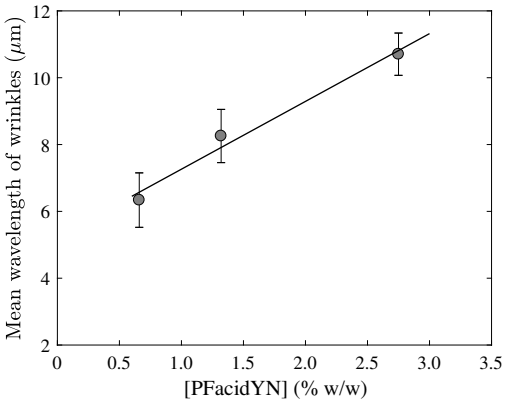

FIGURE 7 – The mean wavelength of wrinkles as a function of concentration of PFacidYN. The error bar is standard deviation from 4∼6 different capsules.

4 Conclusion

Monodisperse chitosan capsules with well-controlled interfacial properties were fabricated by T-shape microfluidic chip. Additional membrane polymerization process in the reaction reservoir which contained desiring concentration of PFacidYN allowed us to tune the membrane shear elastic modulus G_s in a facile way. Within small deformation, D_∞ < 9%, the G_s varied linearly when increasing the membrane complexation time and concentration of PFacidYN, whereas a plateau of G_s value appeared under high concentration of chitosan. Wrinkles and folds on the surface of capsule were also observed. Different from polysiloxane capsules in shear flow, chitosan capsule in elongation flow exhibited eye-catching wrinkles that made it possible to calculate the wavelength.

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