Can sonication increase the release from alginate capsules?

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Résumé :

L'objectif est d'étudier l'influence de la sonication sur les propriétés mécaniques et de relargage de capsules faites d'une membrane souple en hydrogel. Si une forte sonication peut conduire à la rupture de la capsule par fatigue de la membrane, aucune influence n'a été mesurée sur ses propriétés mécaniques en deçà du seuil de rupture. Le relargage est étudié en soniquant des capsules d'alginate remplies de bleu de dextran, mises en suspension dans une solution aqueuse. On montre que le saut de relargage induit par la sonication est proportionnel à la durée de sonication et à la différence de pression ultrasonore. Le relargage passif après une sonication à faible intensité est en moyenne identique à celui mesurée sur des capsules non-soniquées. Ces résultats suggèrent que la stimulation ultrasonore à haute fréquence induit de forts gradients de pression sur la capsule qui conduisent au relargage des molécules encapsulées. La sonication pourrait aussi être responsable d'une augmentation de la porosité de la membrane de la capsule ; ce phénomène serait alors réversible, car la membrane retrouve ses propriétés physiques et mécaniques après sonication.

Abstract :

The objective is to investigate the influence of sonication on the mechanical and release properties of capsules made of a soft membrane in hydrogel. If high sonication may lead to the capsule rupture induced by a fatigue phenomenon, no influence of sonication is measured on the capsule mechanical properties below the breakup threshold. The release is studied by sonicating capsules filled with blue dextran suspended in an aqueous solution. The step in mass release that results from sonication is found to be proportional to the duration time and pressure pulse of sonication. The passive release subsequent to a low-intensity sonication is on average identical to the one measured on non-sonicated capsules. From these results, one can hypothesize that the high frequency ultrasonic stimulation leads to high pressure gradients on the capsule that drive out the encapsulated molecules. Sonication could also lead to a higher porosity of the capsule membrane; this phenomenon would be reversible, as the membrane overall recovers its physical and mechanical properties after sonication.

Key words:　**alginate capsule, sonication, blue dextran release, capsule breakup**

1 Introduction

Ultrasonic stimulation of microbubbles is routinely used in the field of medical imaging. Microbubbles (< 2 microns in size) have been found to be natural innocuous contrast agents, when stimulated by ultrasounds [1]. As the bubbles need to be coated by a lipid layer to be stabilized, they can simultaneously serve as drug vectors for targeted delivery [2]. The drug release results from the large oscillations induced by the ultrasonic stimulation in the gas core [3]. The efficiency of encapsulated bubbles as drug carrier is, however, limited owing to the small quantity of active material that can be carried in the shell and their short lifetime [4]. Liquid-filled vectors are a good alternative, as drugs are typically aqueous solutions. But, if larger quantities of drugs can be encapsulated, the release mechanism induced by ultrasonic stimulation needs to be established for each vector type.

A few studies have tested the effect of sonication on liquid-filled vectors. Sonication corresponds to the exposure of objects such as cells to high-frequency ultrasonic waves, which can possibly lead to their disruption. Schroeder et al. [5] have sonicated liposomes, which consist of a lipid bilayer surrounding a liquid core. They showed that transitory reversible pores were induced by sonication, which led to an

increase in the encapsulated drug release. They found that sonication at low frequency (20 kHz) is more efficient than at high frequency (> 1 MHz) and that the higher the ultrasonic power density, the larger the drug release. The same results have also been obtained on Pluronic micelles, that are spherical structures with a lipid monolayer [6]. Capsules are another type of vector, which consist of a solid membrane around a liquid drop. The only capsules that have been tested under sonication are submicron-sized capsules with a rigid membrane. Shchukin et al. [7] showed that a low-frequency stimulation (20 kHz) could lead to the capsule breakup: the drug release is then induced by the rupture of the capsule shell.

No study has yet considered the effect of sonication on soft-membrane liquid-filled capsules. Our present objective is to measure the effects of sonication on polyelectrolyte capsules in order to understand the release mechanism that can be induced by sonication. As a first exploratory study, we will test millimetric capsules and measure the evolution of the physical and mechanical properties varying the sonication parameters. The release properties will be investigated on capsules filled with a 0.5% blue dextran solution.

2   Materials and methods

2.1   Preparation of liquid-core alginate-membrane capsules

A new fabrication method has been designed to produce calcium alginate capsules. It is inspired from the fabrication processes of Nigam et al. [8] and Nussinovitch et al. [9], the hydrogel membrane capsules being obtained by extrusion in a one-step process. A solution containing 40% (w/v) sucrose of molecular weight 342.3 Da (84100, Sigma Aldrich, USA) and 0.5% (w/v) calcium chloride serves as the liquid core of the capsule (solution A). Sucrose is used as a non-gelling polymer to constitute the core of the calcium-alginate capsules and ensure their spherical shape. We chose to use sucrose similarly to Nussinovitch et al. [9] and not dextran like Nigam et al. [8], because sucrose has a much smaller molecular weight than dextran; it can therefore be washed off more easily from the capsule core, once the membrane is created.

The solution is extruded through a 24 gauge needle (Fisher Scientific) by a peristaltic pump (Ismatec ISM834C, Switzerland) at a flow rate of 1 ml/min. Droplets of solution A form at the tip of the needle and fall into a 0.2% alginate (A0682, Sigma Aldrich, USA) solution (solution B). A distance of 3 cm between the tip of the needle and the surface of solution B ensures spherical droplets. The alginate molecules contained in solution B immediately react with the calcium cations of solution A at the droplet interface leading to the formation of a hydrogel membrane. The reaction time determines the thickness of the membrane. After 5 minutes, the reaction is stopped by a five-fold dilution of the alginate solution with distilled water. The small size sucrose molecules contained in the capsule core are then cleared off washing the capsules with a large volume of distilled water [10]. The capsules are stored in a 0.5% (w/v) calcium chloride solution (solution C), isotonic with the internal liquid, to stabilize the gel membrane. Tests are conducted after one day of storage.

To fabricate blue dextran filled capsules, blue dextran of molecular weight 2000 kDa (D5751, Sigma Aldrich, USA) is added to solution A at a concentration of 5 mg/ml. The capsule fabrication method remains identical for the rest. The capsules are stored in a modified solution C containing the same concentration in blue dextran as the core solution in order to avoid its diffusion. Before use, the capsules are washed with distilled water to clear off the blue dextran molecules sticking on the capsule surface.

2.2   Capsule dimensions

The capsule dimensions are obtained capturing images of the capsules with a CCD camera (JAI M50, Imasys S.A., France). The images are acquired with the Scion Image software (Scion Image, Scion Corporation, USA) and analyzed with Image J 1.42q (National Institutes of Health, USA). The capsules present a very small departure from sphericity. We determine their initial height D_0 and width L_0 on the images to calculate their volume. The average equivalent radius is defined as the radius of the sphere having the same volume. On average, the capsules have a mean radius $r_0 = 1.35 \pm 0.01$ mm. As the capsule membrane appears as more opaque than the liquid core on the images, its thickness can also be determined. The capsules have an average thickness $h_0 = 0.20 \pm 0.01$ mm.

2.3   Capsule sonication

A 30 kHz ultrasonic generator (UP50H, Hielscher, Germany) is used with a 7 mm sonotrode (MS7, Hielscher, Germany). The parameters of sonication varied in the study are the duration time t_s and power P_s

of the ultrasonic stimulation. The sonication power can be adjusted by changing the oscillation amplitude of the sonotrode. The sonication time is ranged from 2 to 35 min and the sonication power from 0.48 to 17.46W.

To determine the influence of sonication on the capsule mechanical properties, three capsules are placed in solution C in a 15 mm diameter tube, itself placed in a water bath to avoid the temperature increase induced by the sonication. The sonotrode tip is immersed in the solution and set 75 mm above the capsules. To determine the influence of sonication on the blue dextran release, samples of 150-350 capsules are sonicated in each test. The total volume of blue dextran solution encapsulated in the capsules is V_{in}. The capsules are placed in a volume V_{out} = 40 ml of solution C in a 28 mm diameter tube.

2.4   Measurement of capsule mechanical properties by compression

The capsule mechanical properties are obtained by compression following the method of Carin et al. [11]. A computer-controlled traction/compression device (Synergie 400, MTS Systems, France) is fitted with a 2 N force transducer (accuracy 10^{-4} N). The capsule is placed on a lower plate within a transparent cup filled with solution C. It is compressed by a piston that moves down at a constant speed. The piston velocity is set at 0.6 mm/min, which is low enough to eliminate inertia effects but large enough to avoid potential osmotic effects. At each time step, the acquisition system records automatically the imposed displacement of the piston $D(t)$ and the resultant force exerted on the piston. The initial contact point between the piston and the capsule corresponds to $D(0) = 0$; it is determined with a precision of ± 20 µm. The buoyancy force acting on the piston is subtracted from the force measured to determine the net force F acting on the capsule.

The capsule mechanical properties are extracted from the experimental curve of the reduced force F/r_0 versus the non-dimensional compression ratio $\delta(t) = (D_0 - D)/D_0$ using the model of Lardner and Pujara [12]. We consider three membrane constitutive laws: the neo-Hookean, Skalak and Evans & Skalak laws (see [11] for more details). Assuming a membrane constitutive law, the apparent dilatation modulus K is found for each value of δ by comparing the theoretical and measured forces. The constitutive law that corresponds to the rheological behavior of the alginate membrane is the one for which K remains constant with δ.

2.5   Measurement of the capsule release

The external solution concentration in blue dextran is measured using a spectrophotometer (SPECORD® S 300 UV VIS, Analytic Jena, Germany) at a 620 nm wavelength. At time t, the release ratio is defined as the ratio of the released mass $m_r(t)$ to the total mass initially encapsulated within the entire capsule sample m_0.

3   Results and discussion

3.1   Effect of sonication on capsule breakup

We have observed that sonication could lead to the capsule breakup depending on the time and power of sonication. The breakup threshold has been investigated varying the conditions of sonication. For each test, the capsule final state (ruptured or unruptured) is determined by naked eye. FIG. 1 shows that the lower the sonication power, the longer the sonication time needs to be to reach capsule breakup. The log-log plot indicates that the breakup threshold follows a negative power law. For a given sonication time, one can expect breakup to occur for sonication powers larger than $P_s^{thr} = 483\ t_s^{-1.7}$.
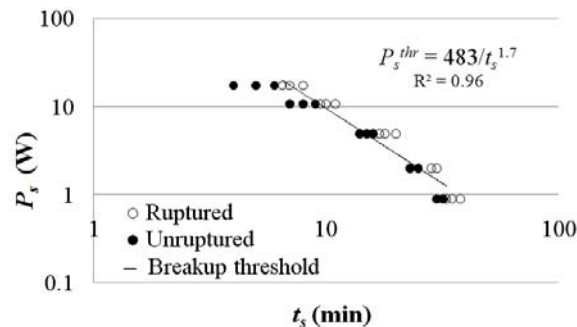

FIG. 1 Diagram of the capsule state at the end of sonication. The full symbols correspond to unruptured capsules and the empty symbols to ruptured capsules. The line is an estimate of the breakup threshold.

The capsule breakup can be attributed to a fatigue effect: it is a consequence of the vibrations generated on the capsule by the ultrasonic field [3, 13]. The vibration amplitude is $(2P_s/\rho cS)^{1/2}/2\pi f$, where ρ is the density of the propagation medium, c is the speed of sound in the medium, f is the ultrasonic frequency and S is the surface area of the sonotrode. The high-frequency vibrations induce a succession of expansions/contractions of the capsule, which eventually lead to the membrane rupture. The ultrasonic cavitation adds up to the fatigue phenomenon and further accelerates the capsule breakup.

3.2   Effect of sonication on the capsule mechanical properties

To analyze the influence of sonication on unruptured capsules, we have measured the mechanical properties of sets of capsules previously subjected to different conditions of sonication. We have first fixed the sonication power at 10.71 W and increased the sonication time up to 9 min (breakup occurs at $t_s \sim 10$ min); we have then increased the sonication power up to 17 W (breakup occurs at $P_s \sim 17.5$ W), setting the sonication time at 6 min. The results are compared with measurements obtained on non-sonicated capsules.

The capsule mechanical properties have been measured using the inverse analysis method of Carin et al. [11]. The different sets of capsules have been tested by compression. The capsules are observed to recover their exact initial shape after two hours, which proves that the membrane is elastic. The small response time is mostly a consequence of the presence of the inner liquid. We find that the neo-Hookean law is the only law for which the apparent elastic modulus K/h_0 is, on the whole, independent of the compression ratio δ; large variations of K are otherwise obtained with the Skalak and Evans & Skalak laws. We find that the alginate membrane follows the neo-Hookean law, whether the capsule is sonicated or not. It proves that the capsule recovers its elastic properties after sonication. FIG. 2 shows the evolution of K/h_0 with the sonication parameters. No significant difference is found between non-sonicated and sonicated capsules, regardless of the sonication time and power. Below the breakup threshold, sonication therefore has a too small influence on the capsule mechanical properties for changes to be detected with the current measurement technique.
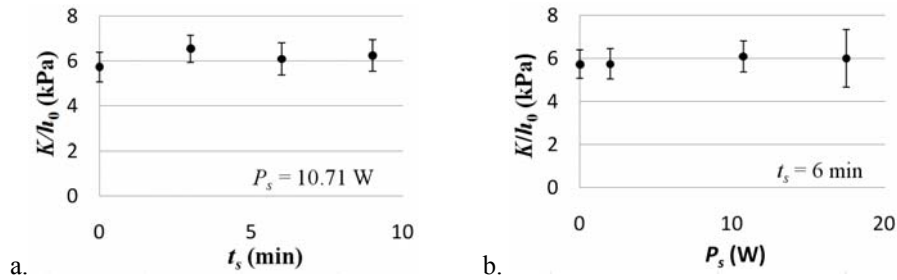

FIG. 2 Apparent elastic modulus obtained with a neo-Hookean law for capsules subjected to different conditions of sonication. $P_s = 0$ W and $t_s = 0$ min correspond to the non-sonicated case.

3.3   Effect of sonication on capsule release

3.3.1   Blue dextran release during sonication

The percentage of encapsulated substance released during the time of sonication is studied for capsules filled with a large molecule of blue dextran. The release step $[m_r/m_0]$ is obtained for different conditions of sonication by measuring the concentration in blue dextran of the suspending solution before and after the sonication. Four sets of capsules are subjected to a sonication power of 0.48 W for duration times ranging from 1 min to 5 min. Another four sets of capsules are sonicated for a fixed duration of 2 min under powers ranging from 0.48 W to 3.06 W. FIG. 3 shows the evolution of the mass ratio released during sonication for the different sets of capsules. The release step is found to increase linearly with the sonication time (FIG. 3a) and the ultrasonic pressure difference $\Delta p = (2\rho cP_s/S)^{1/2}$ (FIG. 3b).

In order to provide a physical explanation for these results, we explore the hypothesis that the mass release step induced by sonication results from the ultrasonic pressure difference Δp that acts on the capsules. Let us model the pressure-driven flow through the membrane as the flow induced through N pores of mean radius r. Under a pressure difference Δp, Poiseuille law predicts the flow rate Q to be equal to

$$Q = \frac{N\pi r^4}{8\mu h_0}\Delta p \ , \tag{1}$$

where μ is the dynamic viscosity of the blue dextran solution. Over the duration time of sonication t_s, the mean flow rate can be approximated by $[m_r/m_0]V_{in}/t_s$. Equation (1) therefore predicts that the release step is

$$\left[\frac{m_r}{m_0}\right] \approx \frac{N\pi r^4}{8\mu h_0 V_{in}} t_s \Delta p .$$
(2)

This simplified model based on Poiseuille law accounts for the linear dependency of the release step with the sonication time and ultrasonic pressure difference. It indicates that the driving force responsible for the substance release through the alginate pores is the ultrasonic pressure difference. This additional pressure, of much larger magnitude than the osmotic pressure, drives the encapsulated blue dextran through the alginate porous membrane. It is also possible that the membrane pore size increases on average during sonication, as the capsule membrane is oscillating.
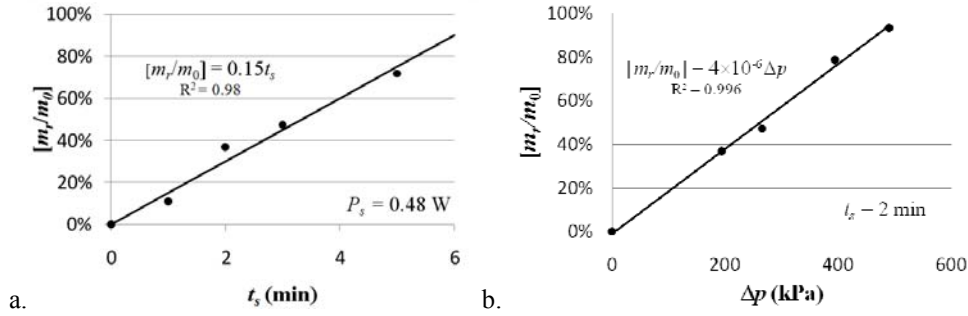

FIG. 3 Step in mass ratio released during sonication measured under various conditions of sonication.

3.3.2 Influence of sonication on the passive release

In order to investigate whether sonication has a permanent effect on the membrane porosity, we finally study the time-evolution of the passive release from loaded capsules. The passive release is measured on two sets of capsules just after their sonication ($P_s = 0.48$ W, $t_s = 2$ and 5 min) and compared to the one measured on non-sonicated capsules (FIG. 4).

Considering first the case without sonication, the curve that best fits the experimental points is

$$\frac{m_r(t)}{m_0} = 0.62\left[1 - \exp\left(-\frac{t}{260}\right)\right].$$
(3)

A nearly perfect fit is found with the exponential law, the coefficient of determination being $R^2 = 0.98$. The blue dextran release therefore tends exponentially towards a constant value of $(m_r/m_0)_\infty = 0.62$ with a characteristic time constant of 260 min. The good fit with the exponential law proves that the release kinetics is diffusion-based: Fick's 1st law, with its assumptions of homogeneous concentrations inside and outside the capsules and time-constant inner and outer volumes, predicts a mass release

$$\frac{m_r(t)}{m_0} = \frac{V_{out}}{V_{in}+V_{out}}\left[1 - \exp\left(-\frac{KA(V_{in}+V_{out})}{V_{in}V_{out}}t\right)\right]$$
(4)

where K is the overall mass transfer coefficient and A the total external capsule surface area available for mass transfer [14]. Where the blue dextran release diverges from Fick's law prediction is in the asymptotic value reached at infinite times. The experimental value of $(m_r/m_0)_\infty$ is much lower than the theoretical value ($V_{out}/(V_{in}+V_{out}) \sim 0.95$). With a molecular weight of 2000 kDa, the blue dextran molecule has a Stoke's radius of ~27 nm, which is slightly bigger than the typical alginate pore size [15]. Previous studies have shown that, in such a case, the encapsulated molecule is only partially released, the asymptotic release ratio decreasing as the relative pore size is decreased [16].

The passive release is then studied on sonicated capsules. FIG. 3a indicates that a 2 min sonication at 0.48 W induces a step in release mass ratio $[m_r/m_0] = 0.33$. FIG. 4 shows that the subsequent passive release has a larger departure from the exponential curve ($R^2 = 0.84$). But on average, the release mass ratio still follows an exponential law: it tends towards the same constant $(m_r/m_0)_\infty = 0.62$ and the characteristic time constant is almost identical (265 s^{-1}). No sensible effect of sonication is therefore found on the passive release properties. For a larger sonication time (5 min), the initial step in mass ratio is $[m_r/m_0] = 0.71$, which is higher than $(m_r/m_0)_\infty$ (FIG. 3a). FIG. 4 shows that no subsequent release takes place once the sonication is terminated. Ahese results show that sonication does not have a remnant effect on the membrane porosity: would it

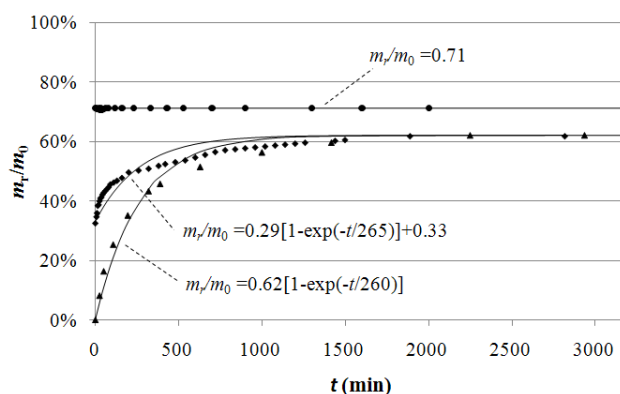increase during sonication, the overall porosity then returns back to its original value after sonication.

FIG. 4 Passive release of blue dextran for non-sonicated capsules (▲) and capsules previously sonicated at 0.48 W for 2 min (■) and 5 min (●). $t = 0$ corresponds to the end of sonication.

4   Conclusion

We have investigated the influence of sonication on capsules with a soft membrane made of hydrogel. No measurable effect has been found on the capsule mechanical properties, as long as the times and powers of sonication remain below a certain threshold. Above threshold, sonication leads to the capsule breakup because of the fatigue of the membrane. When a substance is encapsulated, sonication leads to an increase in the mass release. The step increase measured during sonication is found to be proportional to the duration time and pressure pulse of the ultrasonic stimulation. This linear dependency can be explained modeling the release as the flow through the membrane pores under the ultrasonic pressure. We have finally studied the influence of sonication on the passive release to detect a possible permanent effect on the membrane porosity. Sonication appears to have no remnant effect, as the capsules recover their initial properties on the whole.

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