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Can sonication enhance release from liquid-core capsules with a hydrogel membrane?

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ABSTRACT

The objective is to investigate the influence of sonication on the mechanical and release properties of hydrogel capsules. A new fabrication process is developed to fabricate millimetric capsules made of a highly-viscous liquid core protected by a thin hyperelastic alginate membrane. At high intensities and/or long exposure times, sonication can lead to the capsule rupture, because it induces fatigue in the membrane. Below the breakup threshold, no remnant effect of sonication is, however, measured on the capsule mechanical properties. The release is studied by sonicating capsules filled with blue dextran suspended in an aqueous solution. The mass release that results from sonication is found to be proportional to the sonication duration time and pressure wave amplitude. A possible physical interpretation is that the acoustic streaming flow induced by the ultrasonic wave enhances convection in the vicinity of the capsule membrane and thus mass release. We have finally quantified the passive release subsequent to low-intensity sonications: it is on average identical to the one measured on non-sonicated capsules. Overall the membrane therefore recovers its physical and mechanical properties after sonication. If sonication leads to an increase in porosity of the capsule membrane, the increase is temporary and reverses back at the end of the ultrasonic stimulation.

1. Introduction

Drug release from polymeric delivery systems responsive to external stimulations is receiving increasing attention in therapeutic medicine [1]. It is used to remotely control not only the rate of drug delivery to efficiently meet the time-evolving needs of patients, but also the site of delivery to induce a targeted delivery. The release rate mainly depends on the sensitivity of the drug vector to the stimulation. Among possible stimulations, one finds temperature change [2], pH [3], light exposure [4], magnetism [5]. External force stimulation is another method to induce release from drug carriers. Zanina et al. [6] studied the influence of shear stress and found that release is only reversible at low shear stress. Lee et al. [7] observed that when compression is applied on drug-loaded alginate gels, the gel reacts like a sponge and the drug is squeezed out of the gel. Such experience has never been conducted on capsules.

Less attention has been paid on ultrasonic stimulation. Three decades ago, it has been suggested that ultrasounds could increase the degradation and permeability [8] of polymeric matrices and hence the release of embedded drugs. Currently, ultrasonic stimulation of microbubbles is routinely used in the field of medical imaging. Microbubbles (<2 μm in size) have been found to be natural innocuous contrast agents, when stimulated by ultrasounds [9]. As the bubbles need to be coated by a lipid layer to be stabilized, they can simultaneously serve as drug vectors for targeted delivery. The drug release results from the large oscillations induced by the ultrasonic stimulation in the gas core. 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 [10]. Liquid-filled carriers offer a good alternative, as drugs are typically aqueous solutions. But, if larger quantities of drugs can be encapsulated, the release mechanism induced by sonication needs to be established for each vector type.

A few studies have tested the effect of sonication on drug release from liquid-filled carriers. Schroeder et al. [11] sonicated liposomes, consisting of a lipid bilayer encapsulating a liquid drug. They showed that sonication induces transitory reversible pores on the liposome, which leads 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. Similar results were obtained on Pluronic micelles, which are spherical structures with a lipid monolayer [12].

Capsules are another type of drug carriers, for which the liquid core is protected by a solid membrane with elastic properties. The membrane can be made of various constituents, e.g. reticulated

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polymers [13], reticulated proteins [14], polyelectrolytes [15]. Their self-release ability is determined essentially by the membrane properties. The use of ultrasonic stimulation to promote the release rate has only been studied on submicron-sized capsules with a rigid polyelectrolyte shell [16]. Drug release only occurs if the particle rigid wall is broken up. Shchukin et al. [16] showed that a low-frequency stimulation (20 kHz) can lead to the capsule breakup and that, for a constant intensity of sonication, the duration time of sonication required for breakup increases with the membrane rigidity. But the particles used can be considered rather as liquid-filled rigid microcontainers than as actual capsules, as capsules intrinsically have a deformable membrane by definition.

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 test millimetric alginate capsules. We measure the evolution of the geometrical and mechanical properties varying the sonication parameters. Kühtreiber et al. [17] has indeed shown that the mechanical properties of hydrogel capsules play an important role on their release properties and that any damage on the capsule membrane might change the release behavior.

The methods used to assess the mechanical properties of capsule membranes are typically based on the measurement of deformation under a well-defined stress; a mechanical model of the capsule deformation is needed to infer the membrane elastic properties from the experimental data. Large artificial capsule can be subjected to a shear force in a spinning rheometer [18]; the technique is, however, limited by the rather low level of mechanical stress that can be applied to the capsules. They can also be squeezed between two rigid parallel plates: both the distance between the plates and the compression force are measured simultaneously. This technique is often used to evaluate a bursting force only [19]. It is, however, possible to also extract the membrane mechanical properties through inverse analysis, combining compression measurements with engineering models [20,21]. The experiments consist in measuring the force needed to subject the capsule to a pre-defined deformation. An analytical or numerical model, based on an assumed constitutive law, is then used to deduce the membrane mechanical properties. In the present study, we will apply this technique following the method developed by Carin et al. [22].

The release properties will then be investigated on capsules filled with a 0.5% blue dextran solution. A few methods exist to evaluate the release of an encapsulated substance. The fluorescence detection method requires the encapsulated fluid molecules to be labeled by fluorescence. The evolution of the fluorescence intensity is measured either in the encapsulated fluid or in the surrounding medium in order to estimate the release [23]. High-pressure liquid chromatography (HPLC), another technique used to detect chemical substances in a mixture, may also be applied to quantify released substances [24]. But the method we have chosen is spectrophotometry, which is the most frequently used owing to its high precision and simplicity of use [25]. The absorbance value is measured with the spectrophotometer at various instants of time in the external solution containing the capsules. The corresponding mass released can be calculated calibrating the measurements on samples of known concentrations.

All the experimental techniques used in the study are described in Section 2. We present the new method developed to fabricate the capsules, as well as those used to expose them to ultrasounds and measure their mechanical properties and release. The results are then presented and discussed in Section 3. We first show the effect of sonication on the capsule mechanical properties and possible breakup, before considering its influence on the release of encapsulated molecules. The release induced by sonication is compared with the one induced by compression. We finally show the influence of sonication on the passive release, to study whether sonication has a permanent effect on the capsule porosity. Conclusions on the influence of sonication on capsule release are provided in Section 4.

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. [15] and Nussinovitch et al. [26]: the hydrogel membrane capsules are likewise 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. [26] and not dextran like Nigam et al. [15], 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 min, the reaction is stopped by a fivefold 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. 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, they 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 by 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 upper piston, 8.0 mm in diameter, is used as reference length scale for the calibration.

The capsules present a small departure from sphericity and tend to be oblate ellipsoids. We find a 9% difference between their initial height D_0 and width L_0 measured on the images. We have then calculated their volume assuming the capsules to be axisymmetric, and their equivalent radius r_0, 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 be directly obtained from the acquired images. The thickness is measured at four distinct locations every 90°, in order to take into account possible thickness variations around the capsule circumference. The average thickness measured on a set of capsules is found to be $h_0 = 0.20 \pm 0.01$ mm ($h_0/r_0 = 14.8\%$).

2.3. Capsule sonication

A 30 kHz ultrasonic generator (UP50H, Hielscher, Germany) with a 7 mm sonotrode (MS7, Hielscher, Germany) is used for the capsule sonication. The parameters of sonication that are 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 17.46 W.

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 any temperature increase induced by 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 about 250 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. Capsule compression

The capsule release induced by sonication is compared to that induced by a more classical stimulation: mechanical stimulation by compression. A computer-controlled traction/compression device (Synergie 400, MTS Systems, France) is fitted with a 2 N force transducer (accuracy 10^{-4} N). A capsule filled with blue dextran is placed on a lower plate within a transparent cup filled with solution C ($V_{out} = 10$ ml). As shown in Fig. 1, 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. We define the ratio $\delta(t) = D(t)/D_0$ as the compression ratio. The buoyancy force acting on the piston is subtracted from the force measured to determine the net force F acting on the capsule.

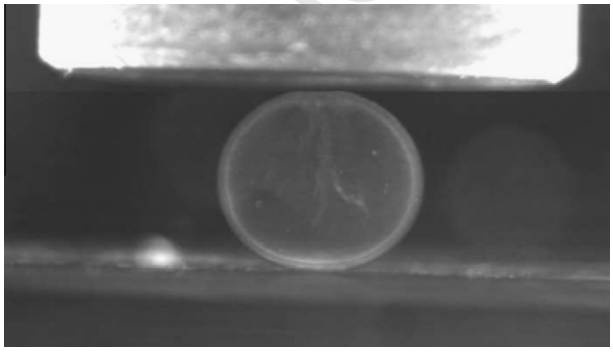

Fig. 1. Compression test of a capsule.

Different values of the maximum compression ratio are imposed: $\delta_{max} = 0.2, 0.4, 0.6, 0.8$. For each test, 30 capsules are compressed one by one in the same external solution of volume V_{out}. The number of capsules tested has been chosen in order to reach values of blue dextran concentrations in the external solution that can be accurately measured with the spectrophotometer. The total volume of encapsulated blue dextran solution in the 30 capsules is V_{in}.

2.5. Measurement of capsule mechanical properties by compression

The capsule mechanical properties are obtained by compression following the method of Carin et al. [22]. Unloaded capsules are tested in the traction/compression device described above. The capsule mechanical properties are extracted from the experimental curve of the reduced force F/r_0 versus $\delta(t)$ using the model of Lardner and Pujara [27]. We consider three membrane constitutive laws, the neo-Hookean, Skalak and Evans & Skalak laws, which correspond to different mechanical behaviors (see [22] for more details). Once a membrane constitutive law is assumed, the surface area-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.

2.6. Measurement of the capsule release

The concentration of the external solution in blue dextran $C_r(t)$ is measured using a spectrophotometer (SPECORD S 300 UV VIS, Analytic Jena, Germany) at a 620 nm wavelength. The mass of blue dextran released $m_r(t)$ is then given by

$$m_r(t) = C_r(t) \times V_{out}. \quad (1)$$

It is to be compared to the total mass initially encapsulated within the entire capsule sample m_0. We will call mass release the ratio $m_r(t)/m_0$. In order to determine m_0, the capsule samples are exposed to a high intensity sonication at the end of each release experiment. The sonication duration time is set in order to guarantee that all the capsules are ruptured. Measuring the concentration C_0 of the solution in blue dextran provides the value of the initially encapsulated mass:

$$m_0 = C_0 \times (V_{out} + V_{in}). \quad (2)$$

3. Results and discussion

3.1. Capsule breakup induced by sonication

We have observed that sonication could lead to the capsule breakup depending on the time and power of sonication. We have therefore investigated the breakup threshold varying the conditions of sonication. For each test, the capsule final state (ruptured or unruptured) has been determined by naked eye. Fig. 2 is a log–log plot of the breakup threshold power P_s^{thr} as a function of the sonication time. Depending on the sonication power, breakup occurs in a matter of minutes (with a maximum of about 1 h). The plot shows that the lower the sonication power, the longer the sonication needs to be to reach the capsule breakup. It also indicates that the breakup threshold follows a negative power law. For a given sonication time, breakup is expected to occur for sonication powers larger than $P_s^{thr} = 483 t_s^{-1.7}$ with P_s in Watts and t_s in minutes. This equation is an estimate of the breakup threshold.

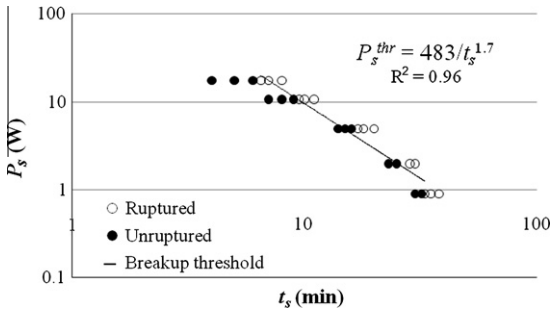

Fig. 2. Diagram of the capsule state at the end of sonication. The full symbols correspond to unruptured capsules and the empty symbols to ruptured capsules. The line is an estimate of the breakup threshold.

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 [28,29]. The oscillation amplitude A_s is a function of the parameters of sonication:

$$A_s = \frac{\sqrt{2P_s/\rho cS}}{2\pi f}, \quad (3)$$

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 vibration amplitude is only of a few microns, but at such a high frequency, it is enough to generate fatigue for the thin elastic membrane of the capsule. The succession of expansions and contractions of the capsule membrane eventually leads to its rupture.

Breakup occurs when stresses are locally greater than the material yield stress. But since stresses and deformations are linked by the local constitutive law, one can also consider that breakup occurs when the total plastic deformation induced during the sonication time t_s reaches a threshold value. For large oscillation amplitudes and above the plastic threshold, the plastic deformation after each cycle can be assumed to be proportional to the amplitude A_s, itself proportional to $\sqrt{P_s}$ according to Eq. (3). Since there are $t_s f$ cycles during the sonication time t_s, the total plastic deformation is hence proportional to $t_s\sqrt{P_s}$. Such simple considerations predict that the breakup power is proportional to t_s^{-2}, which is close to our observation. From the smaller exponent presently found, one may infer that the plastic deformation is actually proportional to the amplitude above a certain yield value.

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); then, setting the sonication time at 6 min, we have increased the sonication power up to 17 W (breakup occurs at $P_s \sim 17.5$ W). The values of the mechanical properties, obtained by compression, are compared with measurements on non-sonicated capsules.

Prior to testing the capsules by compression, we have measured their membrane thickness to see whether sonication had an influence on it. The thickness is found not to change following sonication, no matter what the parameters of the ultrasonic stimulation are. We have therefore considered all the capsules to have a thickness $h_0 = 0.20 \pm 0.01$ mm.

When the capsules are subjected to compression, they are all observed to recover their exact initial shape after 2 h without any creeping effects. It proves that the effect of compression is reversible and that the membrane has elastic properties. The small response time is mostly a consequence of the presence of the inner liquid.

To post-process the data curves obtained from the compression tests, we have used the inverse analysis of Carin et al. [22]. We have calculated the surface area-dilation modulus K as a function of the compression ratio for the three constitutive laws. We find that the neo-Hookean law is the only law for which it is, on the whole, independent of the compression ratio; large variations are otherwise obtained with the Skalak and Evans & Skalak laws. The capsules are therefore strain-softening. We find that the capsule 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. 3 shows the evolution of the apparent area-dilation modulus K/h_0 with the sonication parameters. For the neo-Hookean law, the area-dilation modulus is equal to the elastic or Young modulus: we will therefore call K/h_0 the apparent elastic modulus. 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.

The alginate capsules are measured to have an apparent elastic modulus K/h_0 equal to 6 kPa on average. Even if no other study had previously characterized the mechanical properties of alginate capsules, the result can be compared with measurements obtained on alginate beads. Millimetric beads prepared with a 2.2% alginate solution were found to have a shear modulus just below 12 kPa, which corresponds to an elastic modulus of 36 kPa [30]. We therefore find coherent values of mechanical properties for the present capsules, which are fabricated with a 0.2% alginate solution: the difference in elastic modulus is a consequence of the very different concentrations in alginate and calcium ions used during both fabrication processes.

3.3. Effect of sonication on capsule release

3.3.1. Blue dextran release during sonication

The percentage of encapsulated mass that is released during the time of sonication is studied for capsules filled with a large molecule of blue dextran. It is denoted $[m_r/m_0]$, where the brackets indicate the step increase. It 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. 4 shows the evolution of the mass ratio released during sonication for the different sets of capsules. We have plotted the mass release as a function of the amplitude of the ultrasonic pressure sine wave

$$p_s = \sqrt{2\rho cP_s/S} \quad (4)$$

instead of the power P_s. It shows that sonication leads to pressures of the order of a few bars. The mass release is found to be proportional to the sonication time (Fig. 4a) and ultrasonic pressure amplitude (Fig. 4b). The mass release is therefore also proportional to the ultrasonic wave amplitude A_s according to Eqs. (3) and (4). These relationships of proportionality indicate that, contrary to other types of stimuli, no threshold value exists for release to be induced by ultrasonic stimulation.

In order to provide a physical explanation for these results, we have explored alternative mechanisms to see which one accounts for a linear dependency between mass release and ultrasonic pres-

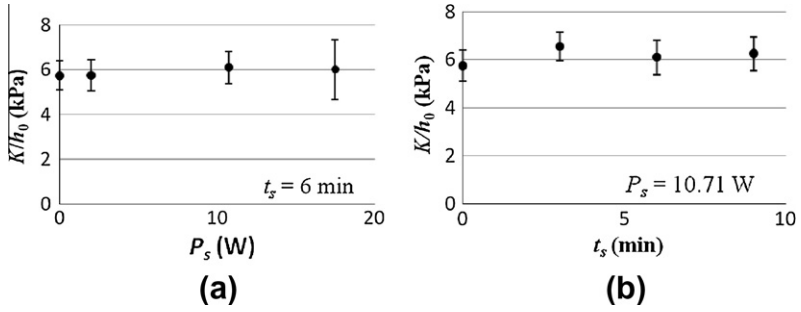

Fig. 3. Apparent elastic modulus obtained with a neo-Hookean law for capsules subjected to different conditions of sonication. P_s = 0 W and t_s = 0 min correspond to the non-sonicated case.

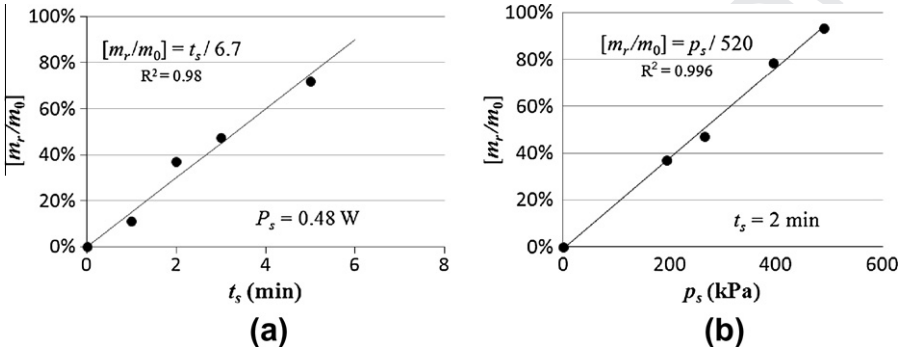

Fig. 4. Step increase in release mass ratio (m_r/m_0) measured at the end of sonication as a function of the time of sonication t_s (a) and ultrasonic pressure p_s (b). P_s = 0.48 W corresponds to p_s = 193.9 kPa. The constants in the regression equations are respectively in minutes in (a) and in kPa in (b).

sure. One mechanism that may drive the internal liquid out is the acoustic radiation force exerted by the acoustic waves on the capsules. If one assumes that the flow through the porous membrane is governed by Darcy's law, one finds that the mass release is proportional to the radiation force. But since the radiation force is proportional to the acoustic power P_s [31], the mass release would then be proportional to p_s^2 (Eq. 4), which is not what is measured experimentally.

Another possible mechanism is the acoustic streaming flow created by the ultrasonic wave. Analytical models have shown that for a narrow sound beam, the streaming velocity U_s is proportional to P_s in laminar flow conditions and to $\sqrt{P_s}$ at infinitely large Reynolds numbers [32]. We have measured the velocity of the acoustic jet to be about 70 cm/s in the case of a sonication power P_s = 3.08 W, which is the maximum power tested in the mass release experiments. The capsules placed in the reservoir are entrained in the large recirculating annular vortex around the jet, where they have a velocity of 5 cm/s. When they occasionally pass under the acoustic jet, their velocity reaches 20 cm/s. The Reynolds number of the flow around the millimetric capsules $Re = 2U_s r_0/\nu$ is therefore of the order of 10^2–10^3, meaning that the flow is laminar (the kinematic viscosity of the suspending fluid is $\nu = 10^{-6}$ m^2/s): the streaming velocity U_s should be proportional to P_s and thus to p_s^2. Such a scaling was found experimentally by Poindexter et al. [33]. The flow around the capsules entrains the liquid by convection within the boundary layer that forms at the capsule surface. The exchange flow induced by convection is determined by the boundary layer thickness. In laminar flow conditions, the latter scales like $Re^{-1/2}$ and hence like $U_s^{-1/2}$ [34]. Since the average mass flux across the boundary layer is inversely proportional to its thickness, $[m_r/m_0]$ scales like $U_s^{1/2}$ and is thus proportional to p_s.

In conclusion, convective effects induced by the acoustic streaming flow account for the linear dependency between the mass release and the acoustic pressure. One can then also easily understand why the release is proportional to the sonication time: the longer the stimulation, the higher the release.

3.3.2. Comparison of the mass release induced by sonication and compression

For comparison, the mass release is measured for loaded capsules subjected to another type of mechanical stimulation: compression. The results are shown in Fig. 5a for different maximum compression ratios δ_{max}. The increase in released mass ratio is directly proportional to the maximum compression imposed on the capsule. The results indicate that, like for ultrasonic stimulation, release takes place even for the low values of compression: no threshold compression value needs to be reached for release to start. The results are also consistent with previous measurements on polyelectrolyte gels: a relationship of proportionality had been found in the special case of gels that are neutral in terms of ion-content with the encapsulated liquid [35].

As the capsule is compressed, the pressure inside the capsule increases. The mass release is induced by the pressure gradient that builds up across the membrane. In order to compare the release induced by sonication and compression, we have plotted in Fig. 5b the mass release versus the pressure difference Δp corresponding to each value of δ_{max}

$$\Delta p = \frac{F_{max}}{S_{contact}}. \quad (5)$$

The force F_{max} is the average net force acting on the capsule at the compression ratio δ_{max}. The contact surface area between the piston and the compressed capsule $S_{contact}$ is obtained measuring the radius of the contact surface on the acquired images. The fact that the mass release is presently found to be proportional to $\sqrt{\Delta p}$ is a consequence of the mechanical behavior of the alginate

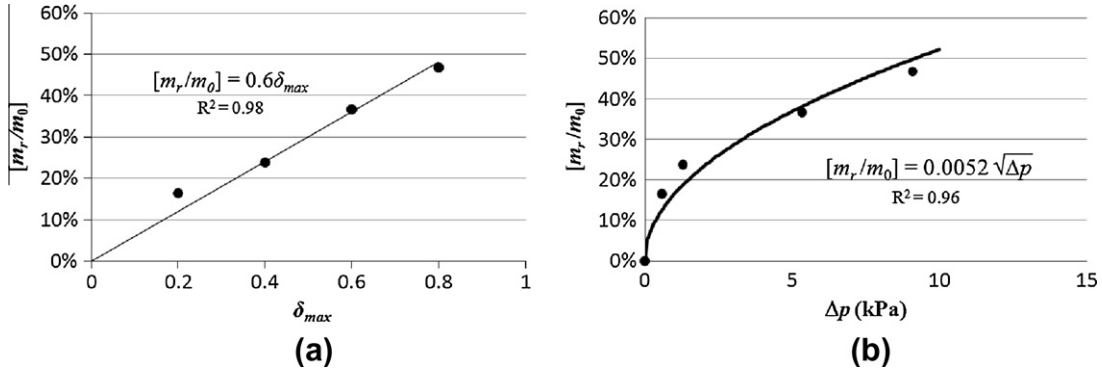

Fig. 5. Step increase in mass ratio (m_r/m_0) released from capsules loaded with blue dextran under compression, as a function of the compression ratio maximum compression ratios δ_{max} (a) and corresponding pressure difference across the membrane Δp (b).

membrane. We have shown in Section 3.2 that the alginate capsule membrane obeys to the neo-Hookean law. A numerical simulation of the compression of a thick-membrane capsule with neo-Hookean properties has indeed proven that the force is roughly proportional to the square root of the compression ratio [21]. One must, however, keep in mind that the driven force (and hence mass release) is lower if the capsule follows a less strain-softening or, even more so, a strain-hardening constitutive law.

To reach a 40% release ratio, the capsule needs to be compressed up to 67% of its initial height. Ultrasonically, the same value of release is reached after a sonication time of only 2 min and 40 s when using the lowest ultrasonic power tested (P_s = 0.48 W, p_s = 193.9 kPa). Two advantages of the ultrasonic stimulation over compression can be highlighted. The most obvious one is that sonication acts on the capsule at a distance and does not require a direct contact with the capsule. Release can therefore be controlled remotely. The second advantage is the very high accuracy that can be achieved when controlling the mass release. Fig. 5a shows that it is difficult to release more than 50% of the encapsulated product by compression. A full release is, however, possible by ultrasonic stimulation.

3.3.3. 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. 6). Considering first the case without sonication, the curve that best fits the experimental points is

$$\frac{m_r(t)}{m_0} = 0.62\left[1 - \exp\left(\frac{-t}{260}\right)\right], \tag{6}$$

in which the time constant is expressed in minutes. 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 toward the constant value of $(m_r/m_0)_\infty$ = 0.62 with a characteristic time constant of 260 min. A similar characteristic time constant has been shown in the case of sodium polystyrene sulfonate incorporated in alginate gels [36]. The good fit with the exponential law proves that the release kinetics is diffusion-based: Fick's first law, with its assumptions of homogeneous concentrations inside and outside the capsules and time-constant inner and outer volumes, predicts a mass release

$$\frac{m_r(t)}{m_0} = \frac{V_{out}}{V_{in}+V_{out}}\left[1 - \exp\left(\frac{-\lambda A(V_{in}+V_{out})}{V_{in}V_{out}}t\right)\right], \tag{7}$$

where λ is the overall mass transfer coefficient and A the total external capsule surface area available for mass transfer [37]. 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 [38]. The membrane therefore limits the blue dextran diffusion, when the molecules travel throughout. 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 [39].

The passive release is then studied on sonicated capsules. Fig. 4a indicates that a 2 min sonication at 0.48 W induces a released mass ratio $[m_r/m_0]$ = 0.33. Fig. 6 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 Eq. (7). It tends toward the same constant $(m_r/m_0)_\infty$ = 0.62 and the characteristic time constant is almost identical (265 min). No sensible effect of sonication is therefore found on the passive release properties of the capsule membrane. For a larger sonication time (5 min), the initial released mass ratio is $[m_r/m_0]$ = 0.71, which is higher than $(m_r/m_0)_\infty$ (Fig. 4a). Fig. 6 shows that no subsequent release or uptake takes place once the sonication is terminated. All these results show that sonication does not have a remnant effect on the membrane porosity, as the overall porosity returns back to its original value after sonication.

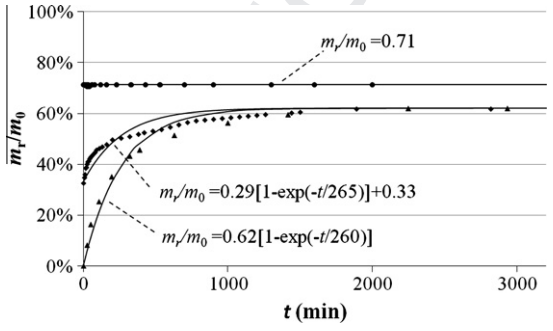

Fig. 6. Passive release of blue dextran for non-sonicated capsules (▲) and capsules previously sonicated at 0.48 W for 2 min (■) and 5 min (●). t = 0 corresponds to the end of sonication.

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 increase measured during sonication is found to be proportional to the duration time and pressure amplitude of the ultrasonic stimulation. This linear dependency can be explained by acoustic streaming: the high-induced velocities enhance convection close to the capsule membrane and thus mass release. If one subjects the capsule to a compression instead of an ultrasonic pressure, we also find a linear relationship of the increase in mass release with the maximum compression ratio. But the comparison proves the higher efficiency of sonication to reach large values of release ratios in short time instants. 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.

The present study proves that ultrasonic stimulation could be an effective method to remotely enhance the release from soft-membrane liquid-filled alginate capsules or induce the capsule breakup. By choosing the sonication time and power adequately, we have shown that it is possible to control the released dose of the encapsulated molecule. A clinical use of sonication appears to be feasible, as previous studies have shown that the range of ultrasonic stimulation presently tested is harmless for cells [40,41]. Sonication could therefore be a powerful external stimulation to trigger a local drug release from loaded polyelectrolyte capsules *in vivo*. For this application, further studies would need to be conducted on smaller size capsules to prove that the results remain valid, as vectorization mainly relies on micro- or nano-capsules. The present results are, however, directly applicable to all the engineering processes that encapsulate fragile or volatile substances and require their release at a precise instant of time. Sonication should then be considered to remotely induced release, as it is a very efficient technique to control a partial or total release within short time durations.

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