Measurement of mechanical properties of alginate beads using ultrasound

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Résumé :

Les billes d'alginate figurent parmi les vecteurs les plus utilisés pour l'encapsulation. Lors de la conception d'organes bioartificiels, elles servent de matrice pour la culture cellulaire. La survie et l'activité des cellules dépendent essentiellement des propriétés physiques et mécaniques des billes. Notre objectif est d'étudier la faisabilité de l'évaluation de propriétés mécaniques de billes d'alginate de taille millimétrique par une technique d'ultrasons. Nous montrons que le premier coefficient de Lamé peut être quantifié et que les billes d'alginate sont formées d'un gel quasi-incompressible. L'étude indique aussi que la masse volumique et le coefficient élastique varient avec la concentration en alginate de la bille et qu'une relation quasi-linéaire existe entre les deux propriétés.

Abstract :

Alginate beads are one of the most common vehicles for encapsulation. When designing bioartificial organs, they are used as matrix for cell culture. Cell survival and activity depend essentially on the bead physical and mechanical properties. Our objective is to study the feasibility of evaluating mechanical properties of millimetric beads using an ultrasound technique. We show that Lamé's first coefficient can be quantified and that the alginate gel is quasi-incompressible. The study also shows that the density and elastic coefficient vary with the bead concentration in alginate and that a quasi-linear relation exists between the two properties.

Mots clefs : **alginate beads, mechanical properties, Lamé's first coefficient, acoustic impedance, ultrasound**

1 Introduction

In the last thirty years, alginate has become the material of choice for encapsulation. It is a naturally occurring copolymer extracted from brown seaweeds that becomes a gel in the presence of calcium ions. It is widely used in bioengineering and in the biotechnology industry owing to its biocompatibility [1]. In tissue engineering, it serves as matrix for cell immobilization [2, 3]. The semipermeable alginate beads allow cell culture in a three-dimensional environment. One potent application lies in the development of extracorporeal bioartificial organs, which consist of cell-encapsulating beads placed in a bioreactor [4, 5, 6, 7]. Alginate beads may also be used for oral drug vectorization. The hydrophilic property of the polymer enables entrapment of water soluble products. By varying the cross-linking of the polymer and the bead concentration in drug, one can control the rate and duration of drug release *in vivo*. Alginate beads can protect other bioactive materials, such as proteins [8, 9], amino acids / peptides [10, 11], hormones [12] or enzymes [13], for gastrointestinal tract delivery systems. Newer fields of application lie in the treatment of vascular diseases. For instance, alginate has been considered as embolic material for endovascular procedures [14] ; entrapment of the vascular endothelial growth factor molecule in alginate beads has shown promising results for therapeutic angiogenesis [15].

Whenever alginate beads are applied to the field of bioengineering, it is necessary to have a fundamental knowledge of the properties of the polymer. In particular, the physical properties of the beads come into play in the mass transfers that occur through the polymeric gel, in the response of the beads to external forces, in their mechanical resistance, etc. When alginate beads are used as a bioartifical medium for cell culture, their mechanical properties play an important role on the cell mechanotransduction. They control the actual stresses applied to the cells and therefore regulate the cell phenotype and activity [16]. Within the bead, the mechanical environment is very different to what is found *in vivo* and strongly varies depending on the type of alginate and the gelation conditions.

Different techniques have already been used to characterize the mechanical properties of alginate beads. Previous studies have always assumed the Poisson ratio ν to be equal to 0.5, therefore assuming the alginate gel

to be incompressible. But none of them validated the hypothesis. They have only characterized one elastic coefficient, either the shear modulus μ (Lamé's second coefficient) or the Young's modulus E, both coefficients being related by

$$\mu = \frac{E}{2(1+\nu)}. \tag{1}$$

David *et al.* [17] measured the shear modulus of millimetric beads, assuming the gel to behave as an elastic material. The beads were tested under compression so as to evaluate the effects of entrapping cells in the beads and of exposing them to a bioreactor environment. The shear modulus of alginate beads entrapping cells was found to be smaller than for empty beads (3 kPa vs 11 kPa), but to increase with the duration of stay in the bioreactor. Atomic force microscopy was also used on slightly smaller empty beads (typically of the order of 0.8-0.9 mm) to determine the effect of the ionic composition on the bead Young's modulus [18]. Values of the Young's modulus were found to be in the same range (1-20 kPa depending on the composition).

One can see that none of the previous studies has actually characterized the Poisson ratio or Lamé's first coefficient λ. The objective of the present study is therefore twofold. First of all, we study the feasibility of using ultrasonic techniques to characterize the mechanical behavior of millimetric alginate beads. We use the pulse-echo method, a non-invasive technique typically used to measure the elastic properties of solid materials. Klemenz *et al.* [19] showed that the technique could be used for alginate gels. They applied the technique on cylindrical samples of alginate and validated the measurement technique by comparing the results with those obtained by scanning acoustic microscopy at high frequency (900 MHz as compared to 6 MHz for the pulse-echo method). In the present study, we show that alginate beads can be tested directly. We then characterize Lamé's first coefficient for the alginate beads and show the influence of the concentration in alginate on the elastic constant.

2 Materials and methods

2.1 Materials

A low viscosity powdered alginate (A0682, Sigma Aldrich, USA) is used in the study. The alginate solution is prepared by dissolving it in a mother saline solution (NaCl 154 mM, HEPES 10 mM). The alginate concentration is varied between 1.5 and 3.5%(w/v). The gelation bath is a $CaCl_2$ solution. A high concentration ($CaCl_2$ 115 mM mixed in the mother saline solution) is used to ascertain a thorough gelation throughout the bead.

2.2 Fabrication of alginate beads

Alginate beads are produced with an extrusion technique [20] (figure 1*a*). The sodium alginate solution is extruded through a 24 gauge needle (Fisher Scientific). A peristaltic pump (Ismatec ISM834C, Switzerland), set with a flow rate of 10 mL/min, is used for reproducibility. The formed droplets fall into a bath of the calcium chloride solution, mixed by a magnetic agitator at low rotating speed to preserve the spherical shape of the beads. They are left to polymerize in the bath for 20 minutes and are rinsed twice with the saline solution. The extrusion process is performed at room temperature. The produced beads are stored at $4°C$ in the mother solution for 48 hours until they are tested : the gelation is then assumed to be complete and stable.

In this study, the aim is to produce beads that are large enough ($\sim$ 2 mm) to be tested ultrasonically. No air co-flow is therefore used during the extrusion. However smaller bead diameters, up to a few hundred microns, can be achieved using the same extrusion technique with an air co-flow. We expect that the results found on millimetric beads will be applicable to any size bead having the same alginate concentration, as long as the level of gelation is kept identical.
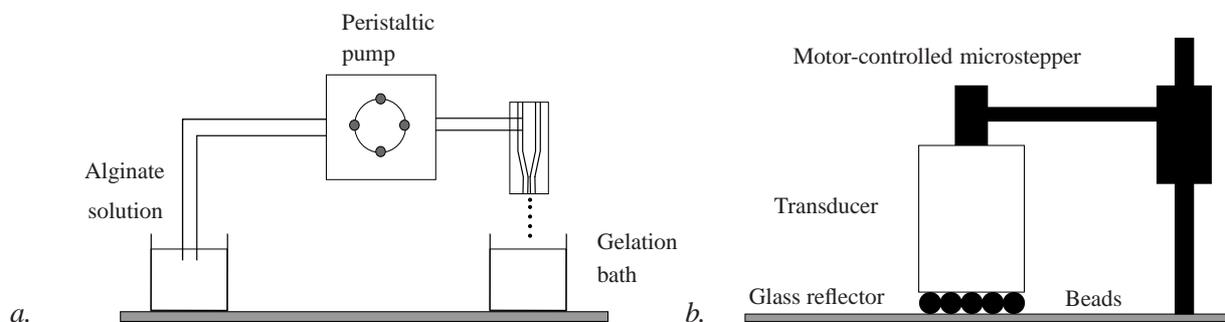

FIGURE 1 – Experimental setups for *a.* the fabrication of alginate beads by extrusion and *b.* the acoustic wave measurement by a pulse-echo method.

2.3 Density measurement

The morphology of the fabricated beads is examined under a calibrated microscope (Leica DM LB, Germany). It is observed that beads formed with low viscosity alginate are perfectly spherical for concentrations above 2%(w/v). Below this value, the beads assume a small tip. We have found that this effect is a consequence of a density smaller for the polymerized bead than for the gelation solution in the case of alginate concentrations below 2%(w/v).

The bead density ρ is measured by first characterizing the bead shape using the microscope. The beads are hypothesized to be axisymmetric and to generally assume an ellipsoidal shape. Their volume is calculated by determining the major and minor axes of the ellipsoid in the plane of view of the microscope. The bead mass is then obtained by weighting the beads with a precision scale (Precisa 92SM-202A, Switzerland). The 0.1 mg accuracy of the scale leads to an error between 1 and 3% in the mass measurement. For each concentration in alginate, the density is measured on seven to ten beads in order to estimate the repeatability of the measurement.

2.4 Ultrasonic measurement

We use a pulse-echo method, also called pulse transmission method. It is based on a piezoelectric transducer that transforms electrical pulses into acoustic waves. The pulse emitted by the transducer travels through the tested material. Part of it is reflected back to the transducer that acts as a receiver once the pulse is transmitted. The received acoustic wave is transformed into an electric signal. The acquired signal is post-processed in order to calculate the acoustic bulk longitudinal velocity c_L and thereafter the compression modulus

$$c_{11} = \rho c_L^2 \tag{2}$$

[21]. The compression modulus can also be expressed in terms of Lamé's coefficients

$$c_{11} = \lambda + 2\mu. \tag{3}$$

The longitudinal velocity corresponds to the velocity of a small-amplitude compressional wave. Since compressional waves only propagate through compressible materials, the alginate gel can be deduced to have a slight compressibility. This result will be proven in section 3.

The classical modus operandi of the pulse transmission technique is to place the sample in water and to measure the acoustic bulk velocity from a unique measurement. The velocity is then determined from the traveling time between two known locations (*e.g.* the time that separates the reflections occurring at the upper and lower surfaces of the sample) that is measured on the signal. Such technique is, however, not applicable in the case of alginate beads, because of their small size and impedance that is too close to the one of water.
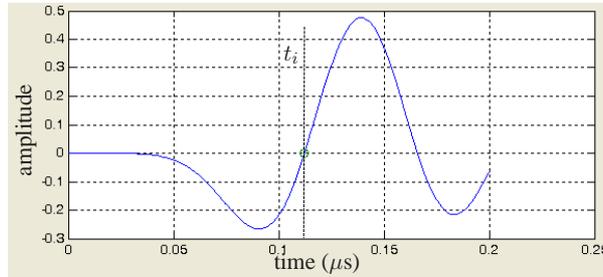

FIGURE 2 – Time-average ultrasonic signal received by the transducer in position z_i ($i = 1, 2$) after transmission through a single layer of alginate beads and reflection on the bottom plane. We choose to compare the difference between the times of the first zero-crossing, t_i. This time difference corresponds to the time during which the ultrasound travels the distance $2(z_1 - z_2)$.

When testing alginate beads, the transducer is instead placed in contact with the beads. Two distinct acquisitions are performed successively. For the first acquisition, the transducer is placed at a height z_1. It is then shifted down by a small distance δz to the height z_2 ($z_2 = z_1 - \delta z$) for a second acquisition. The beads are hence tested under compression. The compression is assumed not to affect the compression modulus, hypothesis that is discussed at the end of this section. On each of the measured signals, we spot the time that corresponds to the first crossing of the time-axis, denoted t_1 on measurement 1 and t_2 on measurement 2 (see figure 2). The difference $|t_1 - t_2|$ is used as the time of travel corresponding to the distance $2\delta z$. The longitudinal wave velocity is then calculated as

$$c_L = \frac{2\delta z}{|t_1 - t_2|} \tag{4}$$

Beads of uniform diameter are placed in a single layer on a plane glass reflector (figure 1*b*). A 10 MHz flat transducer (Panametrics), 12.7 mm in diameter, is connected to the ultrasound generator (Panametrics 5052 UA). It is placed in contact with the bead layer and parallel to the reflector. Accurate displacements are obtained through a motor-controlled microstepper (1 μm in precision), controlled by a custom-made program (Cactus, Roberval Laboratory, Université de Technologie de Compiègne). The reflected signal is visualized using a digital oscilloscope (Lecroy 9410), time-averaged in order to eliminate the noise and acquired by Cactus. A 4 GHz time sampling is used to ensure accuracy in the time measurement ($|t_1 - t_2|$ is of the order of $10^{-8} - 10^{-9}$ s). With a Matlab program, we determine the times t_1 and t_2 on the measured signals and calculate the acoustic bulk velocity.

The measurements are repeated on five different sets of beads issued from the same fabrication batch. The transducer is initially placed in contact with the bead bed and lowered by incremental steps of 0.1 mm. For each measurement set, six acquisitions are done at subsequent positions providing five measurements of the longitudinal velocity. The effect of compression on the acoustic velocity (and *ipso facto* on the compression modulus) is therefore tested on each sample. For compression below one quarter of the maximum diameter, no effect has been observed on the measured acoustic velocity.

3 Results and discussion

3.1 Density of alginate beads

The values of the density measured for alginate beads of increasing alginate concentration are presented in table 1. We observe that the bead density increases with the concentration in alginate. The same result was found by [19], although their values of density were smaller, which is probably due to the type of alginate used to produce the beads. A $2 - 6\%$ accuracy of the measurements has been found, which is reasonable considering the small size and mass of the beads.

Alginate concentration ($\%$(w/v))	Average density (g/cm^3)	Standard deviation ($\%$)
1.5	1.060	4.96
2.0	1.089	2.12
2.5	1.096	2.21
3.0	1.102	2.74
3.5	1.123	6.11

TABLE 1 – Average density of alginate beads of different concentrations with the standard deviation in percentage

3.2 Acoustic velocity and elastic properties

The acoustic bulk velocities, calculated from the acquired signals with equation (4), are shown in table 2. One can see that the longitudinal velocity increases with the alginate concentration of the beads. With a standard deviation below 1%, the pulse-echo method offers a very good accuracy. The results can be compared with those of Klemenz *et al.* [19], who previously characterized beads of lower alginate concentration (1-2$\%$(w/v)) ultrasonically. For a 1.5$\%$ concentration, they found an acoustic velocity of 1500.6 m/s (*i.e.* 0.76% discrepancy with the present result) and 1528.4 m/s at a 2$\%$ concentration (*i.e.* 0.09% discrepancy). Therefore the measured results fall within the error bar of the experimental technique.

Alginate concentration ($\%$(w/v))	Average longitudinal velocity (m/s)	Standard deviation ($\%$)	Compression modulus (GPa)
1.5	1512.0	0.56	2.424
2.0	1527.0	0.57	2.539
2.5	1533.0	0.78	2.577
3.0	1544.5	0.76	2.628
3.5	1554.9	0.63	2.716

TABLE 2 – Average longitudinal sound velocities for alginate beads of different concentrations with the standard deviation in percentage ; corresponding compression modulus.

The compression modulus, calculated from the measured acoustic velocity using equation (2), is indicated in table 2. One can see that the modulus c_{11} is of the order of 10^9 Pa, whereas the shear modulus μ was found to

be of the order of 10^4 Pa by previous studies. Three conclusions can be drawn from this result :
– $\lambda \gg \mu$ in the case of alginate gels, which had already been shown for Agar-gelatin gels by Catheline *et al.* [22].
– Equation (3) can be simplified to $c_{11} \simeq \lambda$, which shows that Lamé's first coefficient of alginate beads can be directly characterized by the pulse-echo method.
– At first order, the Poisson ratio of alginate beads is equal to

$$\nu = \frac{1}{2}(1 - \frac{\mu}{\lambda}), \tag{5}$$

where the correction factor μ/λ is of the order of 10^{-5}. From a mechanical point of view, the alginate gel can therefore be considered as 'incompressible'. However, acoustically, one should speak of 'near incompressibility', since compressional waves can still propagate.

Table 3.2 also shows that the compression modulus (or Lamé's first coefficient) is influenced by the alginate concentration. In order to estimate whether a relationship exists between properties of the beads, we plot the compression modulus of the beads of various alginate concentrations as a function of the corresponding density (figure 3). Although not perfectly linear, a relation of proportionality is observed between the density and Lamé's first coefficient of the beads. The two physical properties are therefore not independent and a equation of linear regression can be determined (presently $\lambda = a\rho + b$, with $a = 4.7$ GPa.cm^3/g, $b = -2.57$ GPa, when λ is in GPa and ρ in g/cm^3).

This result opens the door to a method of characterization of Lamé's first coefficient of alginate beads, that relies only on the measurement of the bead density and the use of a predetermined linear regression between the density and the elastic coefficient. This method bears the great advantage of being simpler to implement than a direct measurement of the Lamé's coefficient by ultrasound.
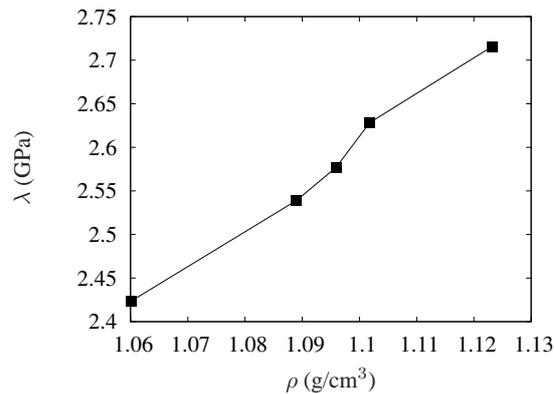

FIGURE 3 – Lamé's first coefficient vs density measured on alginate beads of various concentration in alginate

4   Conclusion

In this study, we have applied a pulse-echo method, which is a non-invasive technique based on the use of ultrasonic compressional waves. We have shown that this technique can be applied to alginate beads of millimetric sizes. Measurement of the ultrasound longitudinal velocity is obtained acquiring the reflected signal for two positions of the transducer, therefore compressing slightly the beads. We have shown that such a technique enables the determination of Lamé's first elastic coefficient of the beads. The coefficient is found to be equal to $2.4 - 2.7$ GPa, so that it is of the same order of magnitude as in the case of water. The gel therefore has a compressibility similar to that of a liquid.

We have tested the influence of the alginate concentration on the density and compressibility modulus of the beads. The results indicate that, within the range of concentrations typically used for cell encapsulation, the modulus increases with the concentration. We have shown that a quasi-linear relationship exists between the compression modulus (or Lamé's first constant) and the bead density. It implies that the determination of the elastic coefficient can be deduced from a density measurement, provided that the regression equation is previously determined for the type of material.

This study enables to complete the characterization of the mechanical properties of alginate beads. Since the beads have been shown to be nearly incompressible, their mechanical behavior is governed by Lamé's second modulus, the shear modulus. Measuring the shear modulus ultrasonically would require using transient elastography, which has never been attempted on samples of millimetric sizes. Hence it seems doubtful at this stage that such a technique can be applied to alginate beads.

Acknowledgements

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