Numerical Modeling of the Shear Module of Alginate Micro-Beads under the Ultrasonic Thermal Effect

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ABSTRACT

The mechanical properties of microscopic particles have been a heated research object for it takes the deformation of micro-beads in the microfluidic environment into account. Sufficient knowledge on mechanical properties of micro-beads would lead to better device design and application for cell mechanics, tissue engineering, etc. The physical properties of alginate beads were examined both in normal condition and under compression, to illustrate its mechanical stability and to calculate the shear modulus through Hertz model. Furthermore, the modeling of physicochemical variation of micro-beads under the ultrasonic thermal effect was performed. The temperature rose simultaneously with ultrasonic thermal effect. The shear module and diameter of micro-beads changed with the increase of temperature in the solution. The descriptive model and the predictive model for the relationship between temperature and the module/diameter of micro-beads were established, and the validation process presented the effectiveness of the models.

1. Introduction

Alginate bead can be made through dropping the alginate solution with different concentrations into chloride calcium solution for the gelling process. This hydrogel plays an important role in biomedical equipments and devices as biomaterials. The beads made of natural materials have advantages over the synthetic beads. The biocompatibility, biodegradability and non-toxicity of natural alginate beads are advantageous [1], whereas the mechanical properties are their shortage. The alginate beads, weakly cross-linked three-dimensional hydrophilic polymers absorbing a large amount of water, can encapsulate various kinds of biological molecules, plant or animal cells as well as different chemical molecules [2].

In the drug delivery system, ultrasonic stimulation can be used as an external stimulation to control drug release from alginate carriers. The effect of ultrasonic stimulation on drug carriers should be investigated. The change in physical properties of alginate bead is valuable in displaying these effects. In the last decades, for the purpose of understanding the mechanical properties of biomaterials, they are exposed to mechanical load such as compression between two parallel rigid plates [3]. Both the stain and full-field displacement are measured. The compression method has been largely applied in the measurement of suspension-cultured cells and microparticles, as well as mammalian cells, yeast and bacterial cells [4, 5]. Normally, three factors, namely the cross-linker, the gelling environment and the polymer

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characteristics, can determine the mechanical properties of alginate hydrogel [6]. It was found that the higher guluronic acid contents and the gelling cations of higher chemical affinityled to stiffer alginate beads [7].

Alginate is a thermally sensitive material. Thermal effect from the environment can change the mechanical properties of alginate gel [8]. This property has also been demonstrated by D Serp [9]. Ultrasonic stimulation as an external stimulation to control drug delivery has a thermal effect on the solution. Thermal effect can be influenced by the power, the pulse mode as well as duration. However, in previous works, the effect of each factor on alginate physical properties has not been studied. Therefore, it is very necessary to investigate the effect of these three key factors on alginate micro-bead mechanical properties.

In this work, the changes of solution temperature with these three key factors are tested; then the thermal effects on the mechanical properties were summarized and numerical modeling was performed to predict the variation of physicochemical properties in alginate microspheres. Residual analyses were conducted to validate the models.

2. Materials and methods

2.1. Alginate micro-beads fabrication

Micro-beads are made of a 2% alginate (A0682, Sigma Aldrich, USA) solution. The alginate solution is extruded through a 24 gauge needle (Fisher Scientific), which defines the bead size, by a peristaltic pump (Ismatec ISM834C, Switzerland) at a flow rate of 1 ml/min. Droplets of alginate solution are formed at the tip of the needle. When the droplet gravity is greater than the adhesion force of the alginate solution, the droplets fall into a 50 ml calcium chloride solution at a concentration of 1.7%. They are left in the calcium solution for 20 minutes so as to polymerize. The distance between the needle tip and the cation solution surface is 10 cm to obtain spherical droplets. The beads are stored in the 1.7% calcium chloride solution to stabilize the alginate gel.

2.2. Ultrasonic stimulation setup

A 30 kHz ultrasonic generator (UP50H, Hielscher, Germany) assembled with a 7 mm diameter sonotrode (MS7, Hielscher, Germany) is used to generate ultrasonic waves. Ultrasonic stimulation duration (ts) is the whole ultrasonic stimulation time, power amplitude (As) is the percentage of ultrasonic stimulation maximal power P, and pulse mode percentage (fp) is pulse work percentage of every second. Three parameters vary in different experiments. For every stimulation experiment, we take six beads as an assay. These beads are put into a tube with 2.5ml 1.7%calcium chloride solution inside and are stimulated under different ultrasonic conditions. The ultrasonic stimulation time is ranged from 2 to 30 min, the ultrasonic stimulation power from 0.48 to 17.46 W and the pulse mode percentage from 20% to 100%. During ultrasonic stimulation, the sonotrode tip is immersed in the solution. The physical properties and the solution temperature for each assay were measured before and after the conduction of the experiments.

2.3. Principle to measure the alginate bead shear module

In order to measure the bead shear module, the compression technology was used. The bead was compressed between two rigid parallel plates and the varying distances between the two plates were recorded. The bead deformation and the compression force exerted on the beads are recorded.

According to the geometrical differences of the samples, different theoretical models were introduced to analyze these experimental data [10]. The Hertz law [11], shown as follows in formula 1, was presented to calculate the shear modulus for a solid spherical bead following the Hooke's law. The compression force variation was expressed for a sphere positioned between two rigid plates, with r_0 as the bead original radius.

$$F(t) = \frac{8\mu r_0^2 \left(\delta(t)\right)^{1.5}}{3(1-\upsilon)} \quad (1)$$

where μ and υ are the shear modulus and the Poisson ratio of the bead material, respectively. Since the Poisson ratio of alginate bead has been found to be 0.5 [12], the force F(t) predicted by Hertz law becomes:

$$F(t) = \frac{16\mu r_0^2 \left(\delta(t)\right)^{1.5}}{3} \quad (2)$$

For every couple of F(t) and δ(t) values measured experimentally, we thus deduce the shear modulus μ.

2.4. Compression test on the alginate bead

A computer-controlled traction/compression device (Synergie 400, MTS Systems, France) is fitted with a 2 N force transducer (accuracy 10-4 N). The alginate bead is placed on the lower plate within a transparent cup filled with1.7% calcium solution, and its shape is continuously monitored by a CCD camera (JAIM150, 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). Contour analysis is performed to detect the bead edge and the initial hight D_0 and width L_0 of the bead. The bead is almost spherical and its volume is computed assuming axisymmetric. An apparent radius r_0 of the bead, which is defined as the radius of a sphere equivalent to the measured bead volume, is 1.12±0.01 mm. The alginate bead is compressed by a piston that moves down at a constant speed of 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 automatically records 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 bead corresponds to D(0)= 0; it is determined with a precision of ±20μm. We define the ratio $\delta = D(t)/D_0$ as the compression ratio. The buoyancy force acting on the piston is subtracted from the force measured to determine the net force F(t) acting on the bead. The variation of F(t) on the function of δ is called the compression curve.

3. Results and discussion

3.1. Measurement of micro-bead shear module and its stability

The compression force F(t) and the deformation δ(t) of an alginate micro-bead, which is conserved in 1.7% calcium chloride solution for 24 hours, are recorded. For every couple of F(t) and δ(t) values measured experimentally, the shear modulus μ(t) is deduced from Formula 1. The shear modulus μ (t) is relatively constant for compression ratios in the range of 5-15%.

Therefore, the experimental shear modulus of the alginate bead is around 42.5kPa. The experimental compression curve has a good fit with the theoretical compression curve.

As the gelation of alginate is a process, the alginate gel needs a certain time to be on the stable state [13]. We prepared five batches of the alginate beads in order to test alginate micro-bead's stability time. They are respectively conserved in 50 ml calcium solution (1.7%) for 20 min, 60 min, 12 hours, 1 days, and 2 days. The shear modulus of the beads is obtained by the method presented above. The measurements are repeated on a minimum of 6 beads for each batch. The shear modules of the alginate beads conserved for different times show that the shear module of alginate bead increases with the conversation time in the first 12 hours and arrives at a stable stat after 24 hours. Other research works report that alginate reaches its stable state after about 15 hours of conversation in calcium solution [14]. Hence, we consider that a gelation time of 24 hours is sufficient to stabilize the alginate gel.

3.2. Thermal effect of ultrasonic stimulation on solution's temperature

In the experiment, ultrasonic stimulation is executed in 2.5 ml 1.7% calcium chloride solution for each six stable beads. A great part of ultrasonic energy is translated into thermal energy, which results in an increase of temperature in the solution. One of the three parameters of ultrasonic stimulation (t_s, A_p, f_s) is varied respectively in different experiments.

When beads are stimulated by A_p=0.2, f_p=0.2 and different t_s, the temperature of the stimulated solution increased linearly as a function of ultrasonic stimulation duration (Fig. 1(a)). The relationship between temperature and duration was established by formula 3:

$$T = 1.526t_s + 20.4 \quad (3)$$

When the beads are stimulated by A_p=0.2, t_s=5min, and different f_s, the temperature of the stimulated solution also increases in a linear manner with the augmentation of the pulse modes percentage f_s (Fig. 1(b)). The relationship between temperature and the pulse modes percentage was established by formula 4:

$$T = 54.526f_s + 20.4 \quad (4)$$

When the beads are stimulated by t_s=5 min, f_s=0.2 and different A_p, the temperature in the stimulated solution also rose in a linear way (Fig. 1(c)). The thermal effects correlated positively with the input power. The relationship between temperature and the power amplitude was established by formula 5:

$$T = 25.456A_p + 20.4 \quad (5)$$

With respect to energy translation, ultrasonic energy is translated into thermal energy and mechanical energy during the stimulation process. In the solution, the increase of the thermal energy is shown by increase of the temperature. It is calculated by function: $Q=CM\Delta T$, where c is the solution specific heat capacity, m is the solution mass and ΔT is the temperature difference. The ultrasound is generated by ultrasound generator. Ultrasonic energy is defined by the generator parameters. The whole ultrasonic energy is calculated by function: W=Psts, where Ps is ultrasonic stimulation power, ts is ultrasonic stimulation duration. Therefore, linear increase of ts, As, and fs means a linear increase of the whole ultrasonic energy w. From the experiment results, we found that all these three parameters have a linear relationship with thermal energy increase in the solution. It is hence considered that a certain percentage ultrasonic energy, which induces the increase of temperature, is translated into the solution. If the linear coefficient is defined as k, the temperature of the solution after ultrasonic stimulation T can be obtained from function:

$$T = k\frac{A_s f_s t_s P}{Cm} + T_0 \quad (6)$$

where T_0 is the initial temperature in the solution.

3.3. Modeling of mechanical module variation

With the increase of temperature, the shear module of the stimulated alginate beads is measured by the compression test. Irrespective of the stimulation condition difference, we found that shear module μ of alginate beads increase with the temperature increase in the solution (Fig. 2 (a)).The module variation as a function of temperature followed the law:

$$\mu = 0.008T^2 - 0.169T + 43.32 \quad [20 < T < 80] \quad (7)$$

R^2 = 0.998 showed good correlative properties for the experimental conditions.

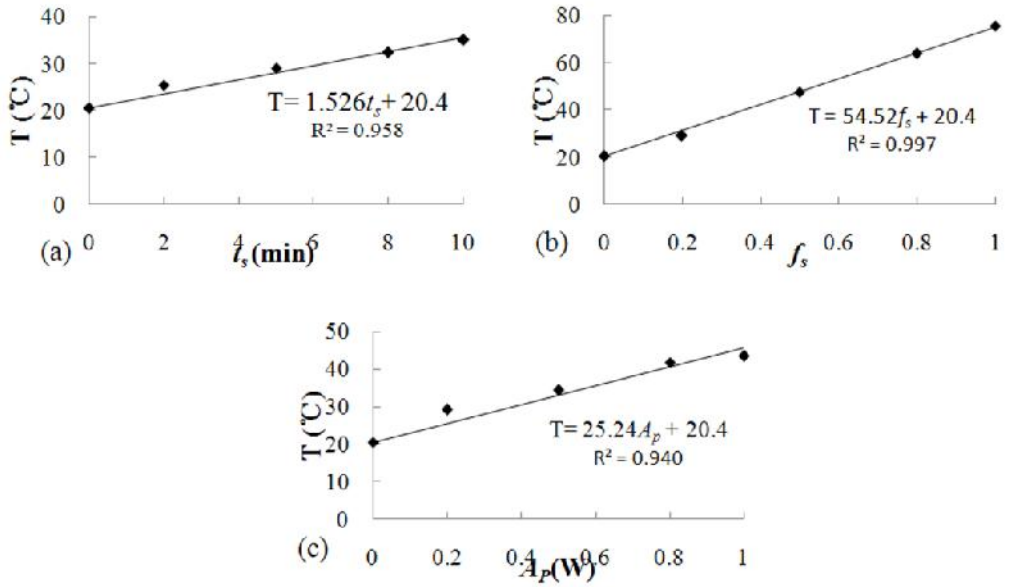

Fig. 1. Temperature of the solution after the stimulation (a) with different t_s and A_p=0.2, f_p=0.2 ; (b) with different f_s and A_p=0.2, t_s=5min ;(c) with different A_p and t_s=5 min, f_s=0.2.

Formula 7 is considered to be the modeling of the shear module depending on the temperature. We further validated its descriptive effectiveness. Through residual analysis of the model, the average relative error is less than 1%, and the experiment conditions fell near the model lines (Fig. 2(b)). The relative error is obtained by calculating the difference between the experimental values and the predicted values of modeling. The residual analysis validated the good descriptive properties of the model.

In order to validate the predictive effectiveness of the model, we stimulated the beads by different conditions, measured the shear module of the stimulated beads and the temperature of the solution after the stimulation. The experimental data are compared with the theoretical data in Fig. 3(a).

As shown in Fig. 3(b), we can see the three points fall in the vicinity of the model curve. Through residual analysis, the average relative error is less than 3.1%, showing the valid predictive properties of the model.

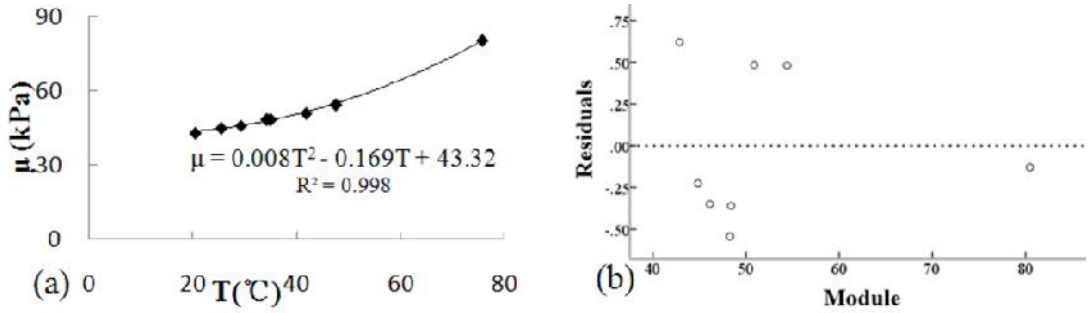

Fig. 2. (a) Variation of the beads shear module with the increase of temperature (experimental data(■) , model curve (—)); (b) Residual analysis of the model to verify the descriptive effectiveness of theoretical formula.

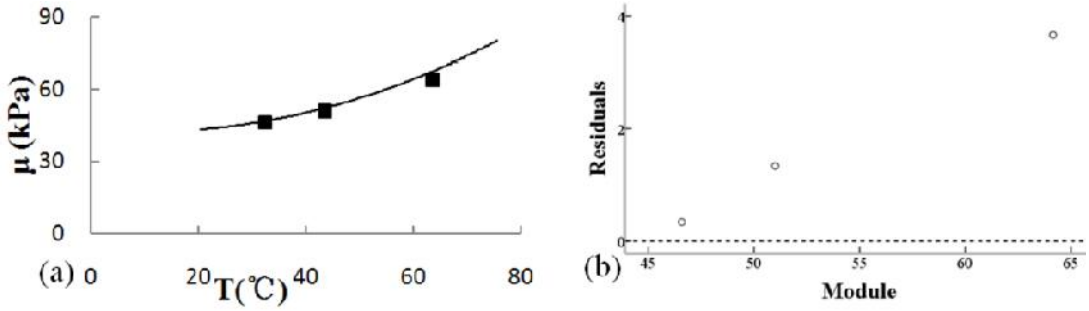

Fig. 3. (a) Validation of the experimental data(■) with the module curve (—) of μ as the function of temperature; (b) Residual analysis of the model to verify its predictive effectiveness.

3.4. Modeling of diameter variation

The relationship between microsphere diameter D and temperature was found to be negatively correlated. With the increase of temperature, microsphere diameter D of the stimulated alginate beads is measured by the compression test. We found that D of alginate beads decreased with the temperature increase in the solution (Fig. 4(a)).The Diameter D variation as a function of temperature followed the law:

$$D = -0.003T + 2.115 \quad [20 < T < 80] \qquad (8)$$

$R^2 = 0.9801$ showed good correlative properties for the experimental conditions.

Formula 8 is considered to be the modeling of diameter depending on the temperature. We further validated its descriptive effectiveness.

Through residual analysis of the model, the average relative error is less than 0.1%, and the points representing experiment conditions fell near the model lines. The residual analysis presented the good descriptive properties of the model in Fig. 4(b). To validate the predictive effectiveness of the model, we stimulated the beads by different conditions, and measured the shear module of stimulated beads and the temperature of the solution after stimulation. The experimental data were compared with the theoretical data in Fig. 5(a).

As shown in Fig. 5(b), we can see the three points fall in the vicinity of the model curve. Through residual analysis, the average relative error is less than 0.5%, showing the valid predictive properties of the model.

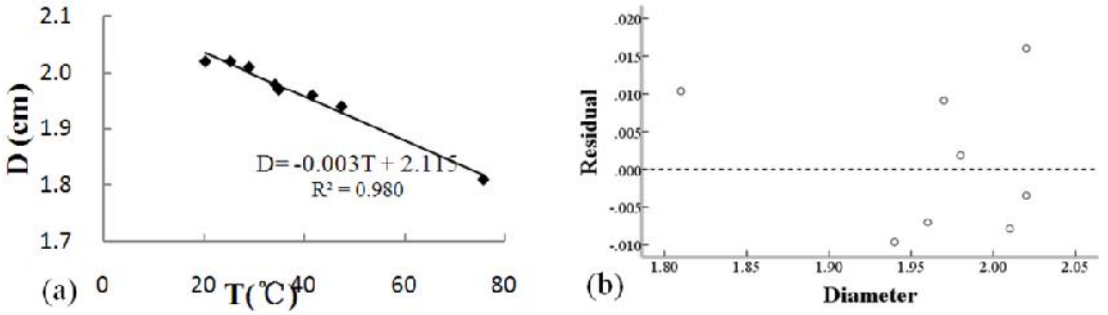

Fig. 4. (a) Variation of the bead diameter with the temperature (experimental data (◆) , model curve (—)) ;(b) Residual analysis on the model to verify its descriptive effectiveness.

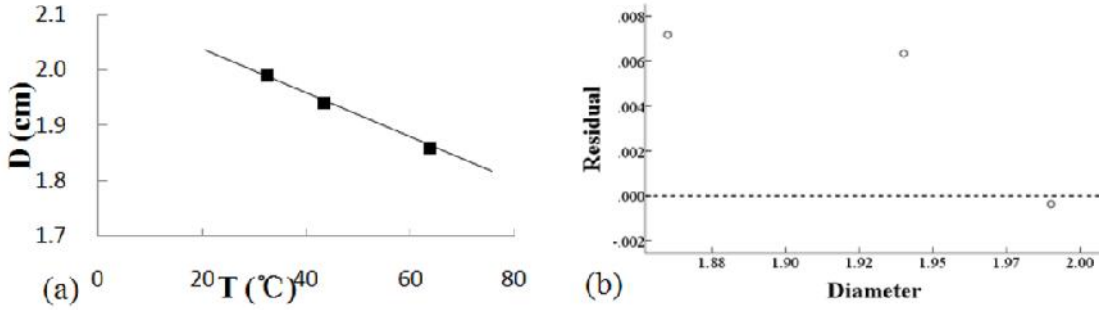

Fig. 5. (a) Validation of experimental diameter data (■) with the theory line (—); (b) Residual analysis on the model to verify its descriptive effectiveness.

4. Conclusions

In this study, the modeling of physicochemical variation of micro-beads under the ultrasonic thermal effect was performed. The shear module of micro-beads conserved in calcium solution (1.7%) was found to be stable in two days; the temperature rose simultaneously with the ultrasonic thermal effects, including ultrasonic stimulation duration (t_s), power amplitude (A_s) and pulse mode percentage (f_p). It correlated positively with each of the three factors. The descriptive model and the predictive model for the relationship between temperature and the module of micro-beads were established, and the validation process presented the effectiveness of the model. The descriptive model and the predictive model for the relationship between temperature and the diameter of micro-beads were also presented, and we validated the effectiveness of this model through different manipulation.

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