

Synthesis and Mechanical Characterization of Chitosan Threads for Biomedical Applications

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Abstract. Chitosan is a biopolymer widely used for many applications, especially in the biomedical field, due to its interesting properties like excellent sorption capability, biodegradability and biocompatibility. The presence of reactive groups in the structure of this biopolymer makes possible the attachment of different molecules. In this study a chitosan thread is synthesized, using a microfluidic technique with two merging channels of acidic chitosan and hydroxide, in order to use it as a controlled release device of drugs. Varying the flows of the fluids in the channels is possible to control the diameter of the thread, obtaining samples of medium and high molecular weight for each flow rate used in the synthesis. The obtained thread of chitosan was mechanically characterized performing tensile tests until failure and stress relaxation tests. An optical system of polarizers was set to visualize the alignment of the polymeric chains while the material is elongated during the tensile test. Young modulus is obtained for each type of sample to quantify the stiffness of the material, and relaxation times were calculated to observe the viscoelastic behavior. Alignment of the material was confirmed, since birefringence was shown.

Keywords: chitosan, thread, microfluidic, mechanical properties, birefringence.

1 Introduction

Chitosan is a lineal copolymer composed of repetitive units of D-glucosamine, which is the predominant unit, and N-acetyl-glucosamine linked by β -(1-4) glycosidic bonds (Fig. 1) [1].

Chitosan is obtained from partial deacetylation of chitin, which is found in the exoskeleton of crustaceans, insects and the cell wall of fungi [2]. The presence of the functional groups amino on C(2) and hydroxyl on C(3), C(6) in the structure of chitosan provides a material with interesting properties like excellent sorption capability, biocompatibility [3], biodegradability. Besides, it is hemostatic, antifungal and antimicrobial agent [1]. These properties allow the processing of the polymer into various geometries as nanoparticles, microparticles, scaffolds, sponges, hydrogels, beads and threads [4]. Some of the applications of the geometries enunciated are as wound dressing, tablet excipient [5], controlled release of drugs [6], gels with antibiotics [7] and gene, protein or peptide entrapment and delivery [11]. The methods

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commonly used for synthesis of the various geometries are techniques based on emulsions, ionic gelation and coacervation/precipitation [10].

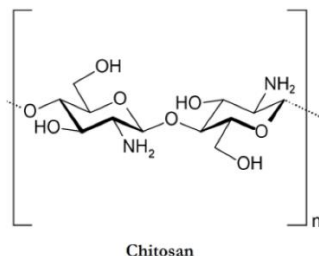


Fig. 1. Chemical structure of chitosan [18].

On the other hand, microfluidics is a technique that manipulates a small quantity of fluid in a chip constituted by an arrangement of channels with dimensions of tens of micrometers. These arrangement of channels allow the processing of the polymer to obtain a certain structure [8]. This technique provides high degree of control over the fluid dynamics, enhanced mass and heat transfer due to the large surface area and improved molecule encapsulation efficiency [9], being suitable for biomedical applications.

In this study a microfluidic technique is used to synthesize a chitosan thread for its future use in biomedical applications as a controlled released device of drugs, employing chitosan of high and medium molecular weight. Tensile tests until fracture and stress relaxation tests were performed to characterize mechanical properties such as stiffness and viscoelastic behavior of the material, respectively.

2 Materials and Methods

2.1 Materials

Medium (190-310 kDa) and high (310-375 kDa) molecular weight chitosan, as well as glacial acetic acid and potassium hydroxide were purchased from Sigma-Aldrich.

2.2 Methods

Preparation of chitosan solution. Chitosan solutions 1.5 % (w/v) were prepared by dissolving chitosan in acetic acid 5 % (v/v) and left 24 hours for deaeration.

Microfluidic device. A microfluidic arrangement was used for the synthesis of chitosan threads (Fig. 2). This device consists in a slide with PTFE tubing (Cole-Parmer, inner diameter 1.42 mm) attached with fast setting epoxy adhesive. Two channels are joined: the first (superior channel of Fig. 2) is connected to the second one through a needle with inner diameter of 0.84 mm. Fig. 2 shows an image of the needle inserted in the channel. The flows employed in the synthesis of chitosan threads are controlled by syringe pumps (Cole-Parmer), one pump for each channel of the microfluidic device.

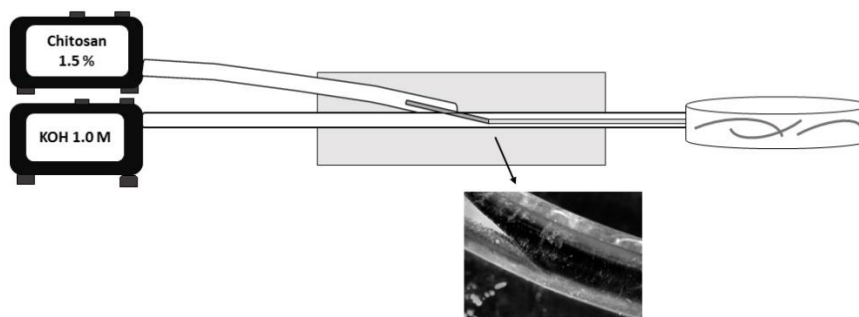


Fig. 2. Microfluidic device. To the left, two syringe pumps used to pump to the device (shown in the center) the solutions of chitosan and KOH. The image shows the moment of exit of the chitosan thread from the needle inside the chip. After the exit, a section of tubing carries the thread to a basic bath.

Synthesis of chitosan threads. For the synthesis of chitosan threads the microfluidic device previously shown (Fig. 2) was used. The main channel contains potassium hydroxide 1.0 M and the secondary channel, a chitosan solution 1.5 % (w/v). Each channel is connected to a syringe pump that controls the flow rates employed for the synthesis according to Table 1.

Table 1. Flow rates used in the main channel (KOH) and the secondary channel (chitosan) for the formation of threads.

Flow rate of chitosan solution (ml/min)	5.0	3.0	0.5	0.3
Flow rate of KOH (ml/min)	1.0	1.0	0.3	0.3

Once the flows are joined, chitosan starts jellifying keeping the diameter established by the needle. The tubing section after the exit from the needle allows the neutralization and gelation of the wall of the thread. This prevents conglomerations of the thread in the collector bath that contains potassium hydroxide 1.0 M. The threads are left in the bath 24 hours, then they are rinsed and kept in deionized water.

Mechanical characterization. The mechanical characterization was performed on a home-made universal testing machine composed by a motorized translation stage and a load cell. An optical system of polarizers was set to visualize the alignment of the structure of 2 cm of chitosan thread while it is elongated during the tensile test until fracture. The samples were put through a progressive and slow strain (rate of 0.03 mm/s). Fracture is achieved and maximum stress and strain values are registered.

Mechanical properties measurements: fundamentals. External forces cause stress on materials. Stress (σ) is defined by the applied force (F) and the cross section (A) of the sample:

$$\sigma = \frac{F}{A}. \quad (1)$$

Changes in the length of the material are quantified by the strain (ε):

$$\varepsilon = \frac{\delta}{L}, \quad (2)$$

where, δ , is the elongation of the sample and L is the original length.

Elastic materials get back to their original length after the applied force ceases, within the so called elastic limit. Stress-strain plots for these materials are straight lines and the slope is the elastic modulus

$$E = \frac{\sigma}{\varepsilon}. \quad (3)$$

In solids, a common assumption when non-linear stress-strain behavior is observed, is a power-law constitutive equation [16].

$$\sigma = E_{nl}\varepsilon^n. \quad (4)$$

In our case, the increment in birefringence suggested that a continuous alignment of polymer fibers is taking place. Before a linear stress - strain regime is observed, this continuous alignment can be fitted by this kind of power-law equation. The same power value “ n ” observed in all the fitting curves, 1.66 in average, fulfills the principle of equipresence required for constitutive equations [17] and gives us confidence in the use of the proposed power-law model.

For the linear stress - strain regime, observed after the non-linear regime, we used:

$$\sigma = E_l\varepsilon. \quad (5)$$

Viscoelastic materials show both elastic and viscous behavior that can be expressed using a time dependent stress function different from the strain function applied. Two characteristic phenomena of viscoelasticity are stress relaxation (stress decreases at constant strain) and mechanical hysteresis (loop in the stress-strain plot obtained from a load-unload test). In a relaxation stress test with a constant strain input, ε_0 , the Maxwell’s model predicts:

$$\sigma(t) = \sigma_0 e^{-\frac{t}{\tau}}, \quad (6)$$

where τ is the relaxation time [14]. For extremely long relaxation times, and based on the serial expansion of the exponential, a linear approximation can be used:

$$\sigma(t) = \sigma_0 \left(1 - \frac{t}{\tau}\right). \quad (7)$$

3 Results

3.1 Synthesis of Chitosan Threads Using a Microfluidic Technique

Eight types of samples were obtained according to Table 2, where it is shown the diameters of the threads depending of the flow rate used in the synthesis. The variation between samples is approximately ± 0.15 mm. Samples synthesized at high flow rates (H-1, H-2, M-5, M-6) exhibit a higher diameter, which indicates a swelling in the thread. Samples synthesized at lower flow rates (M-8, M-7, H-4, H-3) exhibit a lower

diameter than the diameter of the needle (0.84mm). The molecular weight does not influence the diameter of the threads, no matter what flow rate is used in the synthesis.

Table 2. Samples obtained from the synthesis of chitosan threads. Samples with prefix “H” are constituted of high molecular weight chitosan and samples with prefix “M” are constituted of medium molecular weight chitosan.

Sample	Flow rate of chitosan solution (ml/min)	Flow rate of KOH solution (ml/min)	Diameter (mm)
H-1	5.0	1.0	0.91
H-2	3.0	1.0	1.06
H-3	0.5	0.3	0.75
H-4	0.3	0.3	0.70
M-5	5.0	1.0	1.02
M-6	3.0	1.0	0.92
M-7	0.5	0.3	0.92
M-8	0.3	0.3	0.70

3.2 Mechanical Characterization

The stress–strain curves show two regions of different behavior as observed in Fig. 3. The first region exhibits a non-linear behavior. The material elongates, and the polymer chains start to align and order. The second region shows a linear behavior with peaks. At this strain, the material is aligned, and the polymer chains start to slide one from another (peaks shown in the curve) until the thread fractures. Samples of high molecular weight (H-1, H-2, H-3, H-4) resist higher stress, possibly due to an increase in the alignment of the polymer chains. This does not occur easily in samples of medium molecular weight. In contrast, samples of medium molecular weight resist higher strains; possibly due to the disorder of the polymer chains, being less fragile than the ordered polymer chains of samples of high molecular weight.

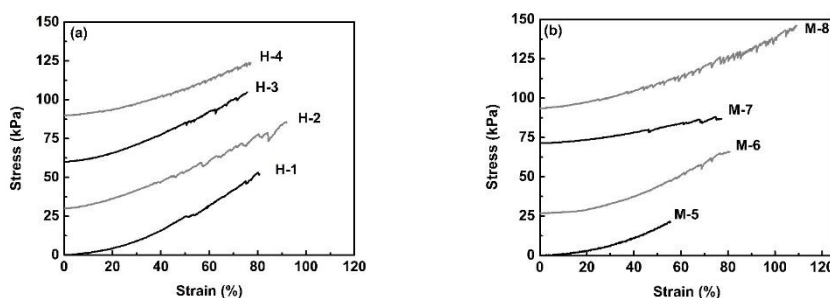


Fig. 3. Stress-strain curves from different samples of chitosan threads. (a) Samples of high molecular weight. (b) Samples of medium molecular weight. Both graphs have a Y offset= 30 between curves.

Table 3 collects all the mechanical properties obtained from tensile tests until fracture. The low variation of the exponent indicates that all samples are constituted from the same material and have the same relaxation mechanism.

Table 3. Mechanical properties of chitosan threads.

Sample	Average maximum stress (kPa)	Average maximum strain (%)	Average non-linear Young Modulus (kPa)	Average lineal Young Modulus (kPa)	Exponent n
H-1	49	68	82	110	1.76
H-2	45	94	58	60	1.57
H-3	46	69	79	90	1.59
H-4	38	82	53	55	1.50
M-5	22	53	52	55	1.75
M-6	34	76	59	60	1.67
M-7	14	53	46	36	1.73
M-8	43	99	55	57	1.67

Average maximum stress values obtained for all samples are consistent with values reported in literature for gelatin hydrogels obtained by microfluidic technique (kPa). Mechanical strength in these cases is bound to the flow used at synthesis, as in our case. It should be mentioned that microfluidic gelatin hydrogels show ultimate tensile strength (12 kPa) and maximum strain (30 %) lower than chitosan threads of this study [19].

Young modulus characterizes the stiffness of the material (Fig. 4). Samples of medium molecular weight are softer materials. Young modulus, linear and non-linear, obtained from the samples do not exceed 60 kPa. The value of the modulus, higher for samples of high molecular weight, suggests that the samples exhibit a more alignment with strain.

Young modulus obtained from chitosan threads of medium molecular weight in this study are consistent with values of Young modulus of 2 % agarose hydrogels (70 kPa), it should be mentioned after agarose hydrogels are modified with PEG-DMA Young modulus increases (90 kPa), as in our case with chitosan threads of high molecular weight (50-110 kPa) [20].

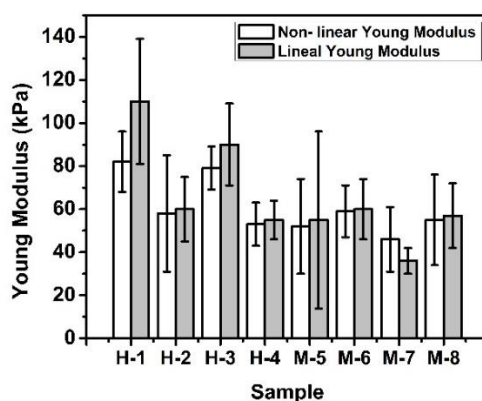


Fig. 4. Young modulus (linear and non-linear) of samples of high molecular weight (H) and medium molecular weight (M).

Alignment of the polymer chains of the material is visualized in Fig. 5. Starting the test, light is blocked by the polarizers; the thread is not illuminated except for a few dots from light reflections. As the thread is elongated it can be observed how light intensity increases, indicating the movement of the polymer chains. Birefringence is shown as the material is elongated. In this case, the material rotates light until it passes through the second polarizer and is caught by the camera.

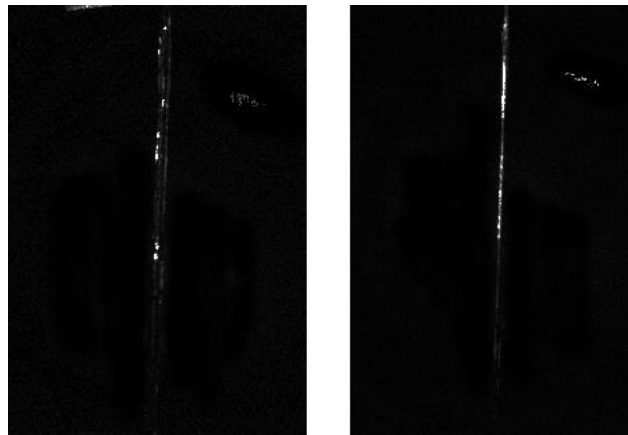


Fig. 5. Visualization of the alignment of the polymer chains while the tensile test is being conducted with crossed polarizers. To the left, image of the start of the test. To the right, image during the test.

Fig. 6 shows low stress relaxation of chitosan threads for both molecular weights; the curve resembles a line. This behavior suggests that relaxation times are extremely long. The curve only shows a small portion of relaxation in the material. The percentage of diminished stress for samples of medium molecular weight is 0.5 % and for samples of high molecular weight is 0.4 %. Relaxation times obtained for samples of high and medium molecular weight were 1322 s and 1150 s, respectively. The viscous component is small. The elastic component is higher than the viscous component.

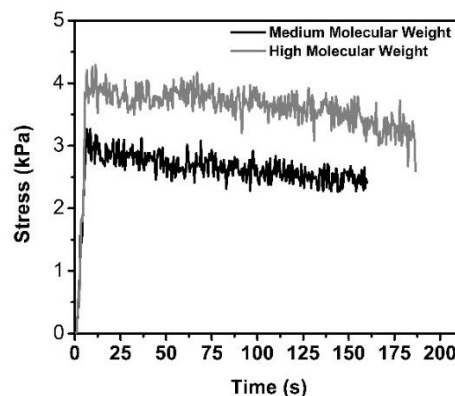


Fig. 6. Stress relaxation graph of high and medium molecular samples.

4 Conclusions

Chitosan threads of high and medium molecular weight were synthesized using a microfluidic technique at different flow rates. The diameter of the threads shows a variation of ± 0.15 mm regarding the diameter of the needle (0.84 mm). The mechanical characterization demonstrates the elastic behavior of the thread and allows the quantification of the stiffness of the material. The stress-strain curves show the viscous and elastic component of the thread. The curve presents two regions, the first region being non-linear and the second region linear. The non-linear region corresponds to the material being elongated and as a consequence polymer chains align. The linear region corresponds to the material already ordered and aligned. The peaks of this region represent the polymer chains giving in and sliding one from another, contributing to the fracture of the material. Birefringence was shown by the material while it was elongated, the alignment of the polymer chains was visualized. Samples of high molecular weight resist higher stresses, while samples of medium molecular weight resist higher strains. The stiffness of the threads vary depending on the molecular weight and the flow rate used at synthesis. The use of fast flow rates and high molecular weight in the synthesis favor the alignment of the material. The values for the Young modulus of samples of medium molecular weight are 45-60 kPa depending on the sample. The Young modulus values of samples of high molecular weight are 50-110 kPa. Relaxation curves show that the material has very low stress relaxation, the percentage of diminished stress is 0.5 % and 0.4 % for medium and high molecular weight, respectively. Relaxation times for high and medium molecular weight were 1322 s and 1150 s, respectively. The viscous component is smaller than the elastic component in this material.

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