Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 34, No. 01, pp. 253 - 261, January - March, 2017
dx.doi.org/10.1590/0104-6632.20170341s20150418

SPINNABILITY, MORPHOLOGY AND MECHANICAL PROPERTIES OF GELATINS WITH DIFFERENT BLOOM INDEX

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(Submitted: July 3, 2015 ; Revised: October 14, 2015 ; Accepted: November 24, 2015)

Abstract - The influence of the concentration, viscosity, electrical conductivity and Bloom index of gelatin solutions on the electrospinning process and the fibrous membrane formed was evaluated. Electrospun membranes were produced at different concentrations for three Bloom values (90, 250 and 280); the solutions were evaluated in terms of viscosity and electrical conductivity and the fibrous membranes by SEM and tensile tests. Results indicate that the electrospinning was most successful when the solution viscosity was within the range of 300 to 700 mPa·s, regardless of the type of gelatin used in the investigated conditions. The average fiber diameter increased with the increased solution viscosity and the relationship between them was well described by a linear equation. With regard to the mechanical properties, GeB250 and GeB280 fibrous membranes showed elongation, on average, at least ten times higher than that of GeB90 membranes.
Keywords: Electrospinning; Biopolymer; Physical characterization.

INTRODUCTION

Natural polymers have been widely studied because they are biodegradable, biocompatible and relatively inexpensive, thereby enabling applications in food, medical and pharmaceutical areas. Gelatin, an example of a natural polymer, has been studied pure or in composite formulas for several applications such as tissue engineering scaffolds (Azhar *et al*., 2014), edible coating (Poverenov *et al*., 2014), adhesives for wound healing (Shefy-Peleg *et al*., 2014), and drug delivery (Phadke *et al*., 2014), among others. In the last decade, researchers have stimulated studies of gelatin applications using electrospinning and producing membranes with specific fiber arrangements (Ki *et al*., 2005; Song *et al*., 2008; Ratanavaraporn *et al*., 2010; Panzavolta *et al*., 2011; Zha *et al*., 2012; Okutan *et al*., 2014). This technique consists of applying a high voltage electric field on a capillary containing polymer solution and a grounded collector plate located at a finite distance from the capillary. The parameters to be controlled in that process refer to the equipment, i.e., the applied voltage, types of collectors, tip to collector distance and feed rate; the ambient parameters (temperature and relative humidity); and finally the solution parameters, such as viscosity, conductivity and surface tension (Bhardwaj and Kundu, 2010). Countless researchers have reported the relationship between these parameters and the spinnability of several polymers (Fong *et al*. 1999; Mit-Uppatham *et al*., 2004; Okutan *et al*., 2014, Bizarria *et al*., 2014). Regarding the solution parameters, they are not only influenced by concentration and composition, but also by the

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polymer properties, mainly molecular weight and ion formation in the solution. Therefore knowledge of the polymer solution characteristics to be electrospun is an important background.

Gelatin may be classified by the type, A or B, and the Bloom index. Gelatin type A is derived from acid-processed collagen, while type B is obtained through alkaline collagen treatment, resulting in products with different isoelectric points: pI 7–9 for gelatin type A and pI 4–5 for gelatin type B (Saxena *et al*., 2005). The Bloom value is the weight, in grams, required for a specified plunger to depress the surface of a standard, thermostatted gel by 4 mm under standard conditions; a higher Bloom index indicates a higher gel strength (Schrieber and Gareis, 2007). In the literature, the relationship between the Bloom index, viscosity, triple helix and molecular weight can be found. The conversion of collagen to gelatin involves disruption of hydrogen bonding and van der Waals attractions between imino residues on different chains, destabilizing triple-helical regions of collagen, leading to a decrease in molecular order (Segtnan and Isaksson, 2004). The gel strength is mainly dependent on the proportion of fractions with a molecular weight of approximately 100000 g mol^{-1}, whereas the viscosity is primarily a function of those with a molecular weight ranging from 200000 to over 400000 g mol^{-1}. For this reason, depending on the method of manufacture, gelatins of the same Bloom value can have quite different viscosities (Schrieber and Gareis, 2007). Bigi *et al*. (2004) report that, in general, the Bloom number is proportional to the triple-helix content of a specific gelatin and the viscosity increases as the higher molecular weight fractions increase. Lai (2009) indicated that high Bloom strength gelatins have higher viscosity than samples with a low Bloom index.

Another characteristic of gelatin is that the higher the Bloom, the lighter the color. The coloration intensity of the gelatin increases with increasing reaction time due to the Maillard reaction between proteins and traces of carbohydrates in the raw material. Other chemical reasons, not yet precisely clarified, can be responsible for the increase in the color intensity of this kind of material. The addition of legally approved oxidizing or reducing bleaching agents such as hydrogen peroxide or sulfur dioxide can also render the product lighter in color (Schrieber and Gareis, 2007).

Researchers have electrospun gelatin by evaluating the type of solvent used (Choktaweesap *et al*., 2007; Song *et al*., 2008; Songchotikunpan *et al*., 2008; Zha *et al*., 2012; Erencia *et al*., 2014), the experimental parameters (Huang *et al*., 2004; Ki *et al*., 2005; okutan *et al*., 2014) and the type of gelatin hydrolysis, acid (type A) or basic (type B) (Ratanavaraporn *et al*., 2010). However, considering the variety of different gelatin molecular structures, few studies evaluate these properties. The influence of Bloom index on mechanical properties of films produced by the casting mode have been investigated by Bigi *et al*. (2004) and, associated wth enzyme immobilization, by Kempka *et al*. (2014). However, this property has not yet been evaluated in the electrospinning process. The aim of this research was to investigate the effect of three different Bloom index gelatins on the spinnability, morphology and mechanical properties of the fibrous membranes produced.

MATERIALS AND METHODS

Solution Preparation

Adequate amounts of gelatin powder type A, from porcine skin, with three different Bloom indexes (90, 250 and 280), kindly supplied by the Gelnex gelatin factory (Itá, Santa Catarina, Brazil), were dissolved in 3 mL of 90% acetic acid (v/v) and stirred at 40 °C for 30 minutes, followed by a subsequent stirring at room temperature for 60 min. In this paper, the gelatin solutions in acetic acid were designated as GeB90, GeB250 and GeB280 in accordance with each Bloom index evaluated. Solutions prepared in various concentrations, ranging from 12 to 32% (w/v) for GeB90, and from 12 to 24% (w/v) for GeB250 and GeB280, were previously characterized by the apparent viscosity at 100 rpm using a Haake 6L Plus viscometer, and by the solution conductivity with a Tecnopon MCA-150 conductivimeter. The polymer and the solvent were used as provided without further purification. All measurements were duplicated at 23.0±0.1 °C.

Electrospinning of Gelatin Solution

Two milliliters of completely dissolved gelatin solution were transferred to a 5 mL glass syringe with a 21 G syringe needle, and electrospun at 10 μL min^{-1} carefully at a controlled flow rate using a syringe pump (N Era Pump Systems NE-300). The applied voltage was 17.0±0.5 kV, and the distance between the needle tip and the grounded collector (Al sheet, 60×60 mm^2) was 17 cm. The temperature was 23.0±0.5 °C, and relative humidity was 55.0±2.0%.

Three categories – bad, good and excellent – were used to classify the spinnability according to the jet

quality produced during the process. A bad classification corresponds to an electrospinning jet breaking up into small droplets frequently or difficulty in ejecting the jet; the good category means fiber formation with a few droplets reaching the collector, and the excellent classification was given to the condition when no droplets were observed in the collector, yielding a homogeneous film.

Microscopy

The morphology of the as-spun fibers was examined by scanning electron microscopy (SEM) (JSM-6390LV, JEOL), and the fiber diameters were determined by performing at least 50 measurements for each specimen to obtain an average value, using the Size Meter 1.1 program (LCP-UFSC, 2012).

Mechanical Properties

The samples submitted to a tensile test were prepared according to Huang *et al*. (2004) and Chen *et al*. (2009). First, double-sided tapes were glued onto the top and bottom areas of one side of a cut white paper template (50x50 mm^2), as shown in Figure 1A. This template was glued onto the fiber mat topside, which was cut into rectangular pieces (10×50 mm^2) along the vertical lines as shown in Figure 1B. Then, the aluminum foil was carefully peeled off and single-sided tapes were applied onto the gripping areas as end tabs.

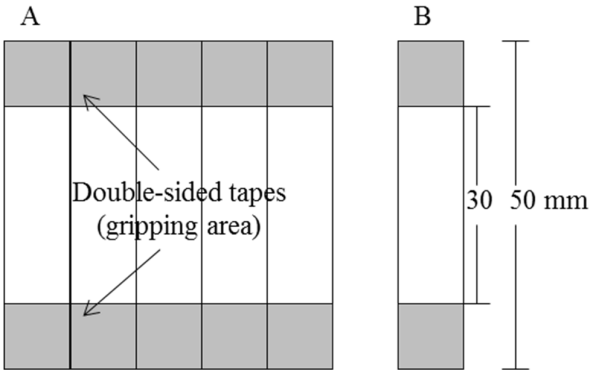


Figure 1: (A) A paper template model used to prepare tensile specimens of the electrospun nonwoven fiber membrane, and (B) a tensile specimen model.

Tensile tests were performed using a texturometer (TA. HD. Plus, Stable Micro Systems) with a load cell of 500 N and displacement rate of 10 $mm.min^{-1}$ at 23 °C. The initial distance between the texturometer grips was 30 mm. The fiber mat thickness was measured by a digital micrometer (Digimess, IP54) and at least five measurements for each material were taken; the average was calculated for each electrospun mat.

RESULTS AND DISCUSSION

Effect of Solution Concentration

The spinnability, electrical conductivity and viscosity of GeB90, GeB250 and GeB280 gelatin solutions in 90% (v/v) acetic acid, at concentrations from 18 to 32%, 12 to 24% and 12 to 24% (w/v), respectively, were evaluated. Table 1 presents the results of conductivity and viscosity, as well as the spinnability quality for all types of gelatin Bloom index. It was observed that conductivity increases as the solution concentration increases, which can be attributed to the increase in the total amount of ions in solution. It must be noted that gelatin is a kind of polyelectrolyte, with ionizable groups such as amine and carboxylic functional groups, susceptible to ionization by acidic agents; these groups can also be hydrolyzed to carry positive or negative charges (Huang *et al*., 2004; Songchotikunpan *et al*., 2008). At the same concentration, the conductivity value increases as the Bloom index decreases; this provides evidence that the lower the Bloom index, the smaller the size of the polymer chains, thereby increasing the number of ionizable terminal groups, such as amine and carboxyl groups.

The viscoelastic property of polymeric fluids has been identified as the most important parameter to be considered in the electrospinning process operation and this property can be well represented by a simple measurement of solution viscosity to indicate the spinnability (Ratanavaraporn *et al*., 2010). The viscosity values of the gelatin solutions were found to increase with increasing gelatin concentration [Ge] (Figure 2a), the result of a greater number of polymer chain entanglements within the solution. It can also be observed that the viscosity values for GeB90 were much smaller than those for GeB250 and GeB280 and the latter were very similar to each other from 12 to 20% (w/v).

The polymer molecular weight represents the polymer chain length. In turn, it has an effect on the solution viscosity since the polymer length will determine the polymer amount necessary to produce polymer chain entanglements in the solvent. It is well known that, by using capillary rheometry, a double logarithmic plot, made for viscosity and concentration of polymer solution, can be fit by two

straight lines with different slopes. For the points at which the two straight lines in the former and the latter plot meet, a critical concentration can be defined (Hayahara and Takao, 1968). The existence of these critical quantities has been discussed in terms of the entanglements among polymer chains that are necessary to maintain the continuity of the jet and the fiber formation during the electrospinning (Gupta *et al*., 2005; Ramakrishna *et al*., 2005; Shenoy *et al*., 2005). Figure 2b show the double logarithmic relationship between apparent viscosity and polymer concentration of gelatin solutions having different Bloom index in 90% (v/v) acetic acid. As can be seen, a single straight line was well adjusted for each gelatin type (Equations, 1, 2 and 3 for GeB90, GeB250 and GeB280, respectively), indicating that the concentration ranges studied are above the critical concentration because there was fiber formation for all solutions evaluated.

$$\log\eta_a = -1.65 + 2.99\log C \quad R^2 = 0.99 \tag{1}$$

$$\log\eta_a = -0.96 + 2.79\log C \quad R^2 = 0.99 \tag{2}$$

$$\log\eta_a = -1.15 + 2.96\log C \quad R^2 = 0.98 \tag{3}$$

The spinnability of GeB90, GeB250 and GeB280 at low concentrations showed bad spinnability characterized by droplet formation, resulting from low solution viscosity (Fong *et al*., 1999; Mit-Uppatham *et al*., 2004). The spinnability improved with increasing concentration of the solution, but at excessively high concentrations, it became worse. At high concentrations, the polymer solution shows a viscoelastic behavior, hindering the jet ejection. Although fibers were formed, a polymer solution instability at the needle tip was observed, and a thick jet was often ejected, reaching the collector without sufficient solvent evaporation. Excellent spinnability was observed for GeB90 in a higher concentration range than for GeB250 and GeB280. GeB90 does not form fibers in the range from 12 to 16% (w/v) and the best concentrations were from 24 to 30% (w/v), in contrast with GeB250 and GeB280, for which the best range was from 18 to 22% (w/v). This behavior confirms that the higher the gelatin Bloom number, the higher the solution viscosity.

If the spinnability is analyzed in terms of the results of viscosity, it is possible to observe that the best viscosity ranges for different gelatin Blooms is similar, i.e., 307 to 512 for GeB90, 322 to 603 for GeB250 and 324 to 666 mPa.s for GeB280. Based on the results shown in Table 1, it is difficult to establish an accurate viscosity range, but they are in agreement with Songchotikunpan *et al*. (2008). They investigated the electrospinning of a gelatin extracted from Nile tilapia (Bloom 328 g) in acetic acid and concluded that droplets were formed when the solution had shear viscosity values lower than 336 mPa.s. They also report that it was difficult to electrospin gelatin solution with 994 mPa.s using 90% (v/v) acetic acid.

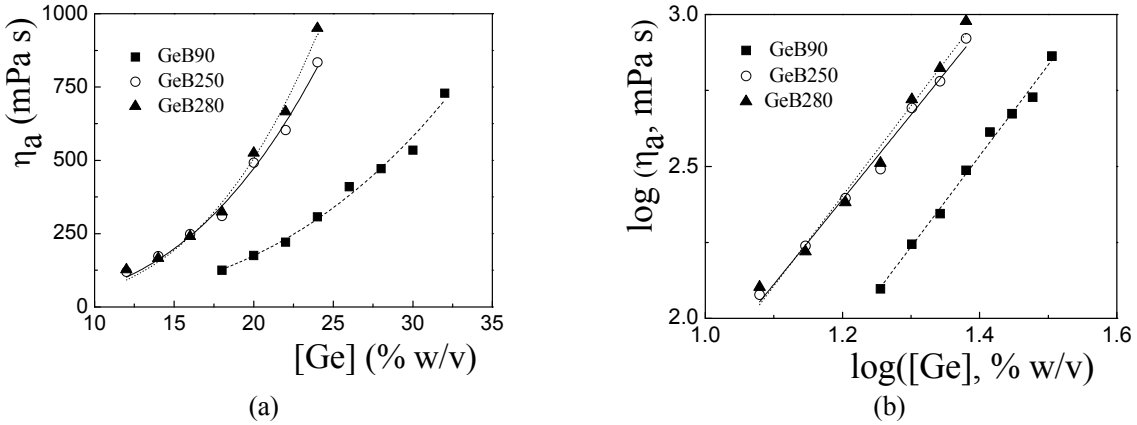


Figure 2: Viscosity as a function of gelatin concentration (a) and log-log plot between gelatin concentration and the solution viscosity (b) for solutions with different Bloom index values.

Table 1: Spinnability, viscosity and conductivity of different concentrations of GeB90, GeB250 and GeB280 solutions in 90% (v/v) acetic acid.

[Ge] (% w/v)	GeB90			GeB250			GeB280		
	Spinnability	Viscosity (mPa.s)	Conductivity (μS/cm)	Spinnability	Viscosity (mPa.s)	Conductivity (μS/cm)	Spinnability	Viscosity (mPa.s)	Conductivity (μS/cm)
12	-	-	-	bad	120±3	452±20	bad	127±4	428±16
14	-	-	-	good	173±6	487±21	good	166±6	462±15
16	-	-	-	good	249±9	515±24	good	241±11	496±26
18	bad	125±3	599±51	excellent	310±4	541±27	excellent	324±13	520±24
20	good	175±5	616±44	excellent	492±6	559±26	excellent	525±18	542±20
22	good	221±12	633±42	excellent	603±20	578±30	excellent	666±8	560±21
24	excellent	307±7	641±43	bad	834±14	597±33	bad	950±27	570±26
26	excellent	410±9	650±45	-	-		-	-	
28	excellent	472±13	657±46	-	-		-	-	
30	excellent	528±4	661±49	-	-		-	-	
32	bad	729±19	669±52	-	-		-	-	

Morphology

SEM micrographs (Figure 3) show the morphology of the good and excellent spinnability from GeB90, GeB250 and GeB280 fiber mats. In addition to interfering in the spinnability, concentration and viscosity also affected the electrospun fiber diameter, i.e., an increase in concentration or viscosity results in thicker fibers. This effect can be attributed to the increase in viscoelastic forces, generated by the increase in the degree of chain entanglements, leading to a stretching decrease of the polymeric jet and resulting in formation of thicker fibers (Li *et al*., 2005; Choktaweesap *et al*., 2007). Figure 4a illustrates the average diameter of electrospun GeB90, GeB250 and GeB280 fibers plotted as a function of the concentration. The average fiber diameter for GeB90 increased from ca. 257.3 to 646.2 nm, for GeB250 from ca. 204.3 to 588.3 nm, and for Ge280 from ca. 222.7 to 630.1 nm. For a given solution concentration, GeB90 presented finer fibers than GeB250 and GeB280, which, from 14 to 18% (w/v), were found to be very comparable to each other. The log-log plots between the solution concentration (C) and average fiber diameters (D), Figure 4b, were well represented by single straight lines, confirming that the electrospinning solution concentration are above the critical concentration (Equations (4), (5) and (6) for GeB90, GeB250 and GeB280, respectively).

$$\log D = -0.388 + 2.15 \log C \qquad R^2 = 0.97 \qquad (4)$$

$$\log D = -0.473 + 2.42 \log C \qquad R^2 = 0.99 \qquad (5)$$

$$\log D = -0.533 + 2.48 \log C \qquad R^2 = 0.95 \qquad (6)$$

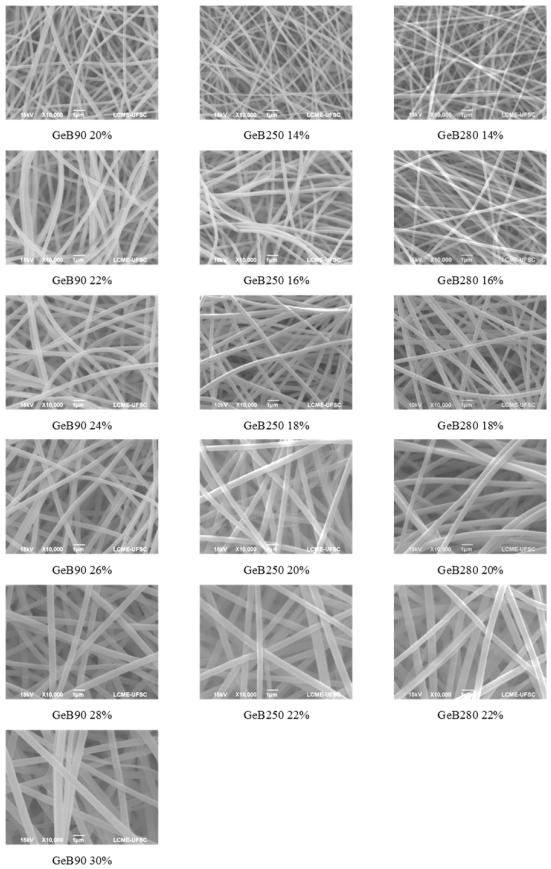


Figure 3: Scanning electron micrographs of electrospun mats. The Bloom index and solution concentration are shown below each image (magnification = 10000×, scale bar = 1 μm).

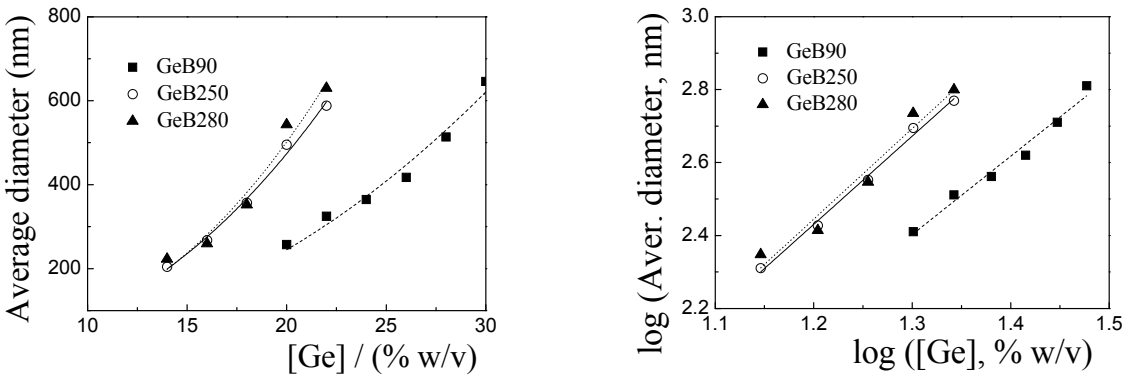


Figure 4: Average diameter of electrospun GeB90, GeB250 and GeB280 fibers plotted as a function of the concentration (a) and log-log plot between fibers diameters and the solution viscosity of the three different gelatins (b).

Figure 5 shows a scatter plot of the average fiber diameter values as a function of viscosity in an associated form. The dispersion suggests that, independent of the Bloom index, there is a common relationship between fiber diameter and solution viscosity, which is well described by a linear equation (Equation (7)).

$$D = 82.3 + 0.88\eta_a \quad (R^2 = 0.94) \tag{7}$$

Mechanical Properties

Mechanical properties of homogeneous electrospun fibrous membranes obtained with excellent spinnability were evaluated by tensile tests (Figure 6), and the average Young's modulus, the ultimate strength and the elongation are summarized in Table 2. From the results for all different types of gelatin, higher concentration ranges produce thicker membranes. The increase in concentration causes an increase in solution viscosity, which is related to the entanglement degree of the polymer chains, as previously mentioned. The analysis of the fibrous membrane thicknesses suggests that, above a certain viscosity there occurs a more stable Taylor cone, promoting better orientation of the fibers towards the collector. Huang *et al*. (2004) showed similar behavior, i.e., concentration of the electrospun gelatin solution resulted in thicker membranes. However, the difference in thickness is minimized in the calculation of the stress and strain parameters (Callister, 2007).

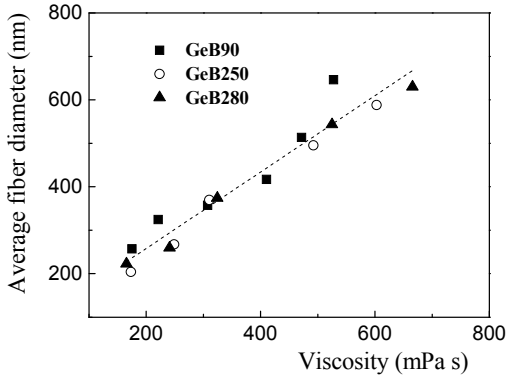


Figure 5: Average diameter of electrospun GeB90, GeB250 and GeB280 fibers plotted as a function of the solution viscosity.

Table 2: Tensile properties of the nanofiber membranes electrospun with gelatin solutions of different Bloom indexes.

[Ge]/ % (m/v)	Thickness / µm	Young's modulus / MPa	Ultimate strength / MPa	Elongation / %
[B90] = 24	55.4 ± 12.7	88.0 ± 25.6	1.5 ± 0.1	1.2 ± 0.2
[B90] = 26	120.0 ± 7.0	80.8 ± 16.1	1.5 ± 0.2	2.0 ± 0.5
[B90] = 28	144.4 ± 1.1	72,0 ± 16,4	1.4 ± 0.1	1.8 ± 0.2
[B90] = 30	126.4 ± 10.4	91.0 ± 12.3	1.6 ± 0.1	1.6 ± 0.3
[B250] = 18	48.6 ± 9.4	55.1 ± 16.6	2.1 ± 0.4	19.7 ± 1.5
[B250] = 20	91.5 ± 2.1	74.1 ± 18.5	2.2 ± 0.3	28.9 ± 8.7
[B250] = 22	89.5 ± 4.1	87.9 ± 8.8	2.4 ± 0.4	23.4 ± 12.4
[B280] = 18	47.3 ± 5.4	49.7 ± 9.4	2.6 ± 0.3	22.6 ± 6.5
[B280] = 20	96.3 ± 5.4	87.4 ± 11,1	2.5 ± 0.2	22.2 ± 9.0
[B280] = 22	100.8 ± 6.1	90.4 ± 18.9	2.3 ± 0.3	18.9 ± 4.7

Different letters in the same column indicate significantly different values by the Tukey test ($p \leq 0.05$).

For the Young's modulus, only the concentration influence is meaningful in the response variability, with a value of determination coefficient of 0.68. However the Tukey test showed no difference at a significance level $\alpha = 0.05$, indicating that, in the conditions studied, no changes are observed in the elastic modulus (Table 3). For the Ultimate strength, only the Bloom index is meaningful in the response variability, with a coefficient value of determination of 0.86 (significant Tukey test for all Bloom values, $p < 0.05$ - Table 4). For the elongation, although the results of analysis of variance were not globally significant, significance was observed for Bloom equal to or greater than 250 compared to 90 (Table 5).

Table 3: Tukey's test applied for the Young's modulus of MF with different Bloom indexes.

Bloom	B90 82.9	B250 71.5	B280 75.8
B90		0.104	0.375
B250	0.104		0.719
B280	0.375	0.719	

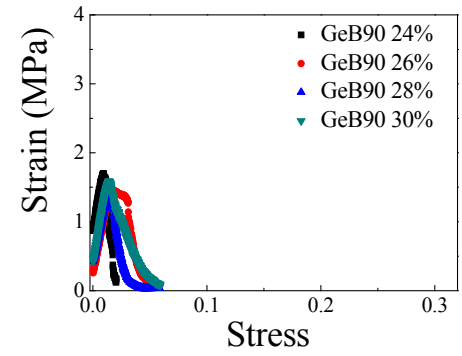
Table 4: Tukey's test applied for the Ultimate strength of MF with different Bloom indexes.

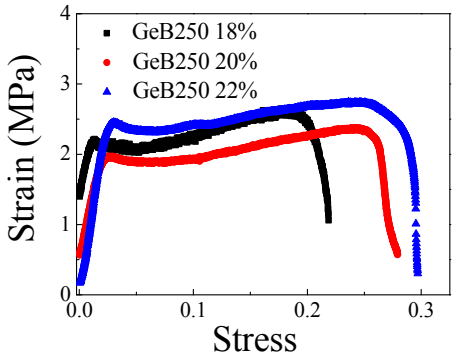
Bloom	B90 1.52	B250 2.22	B280 2.47
B90		**0.000128**	**0.000128**
B250	**0.000128**		**0.0321**
B280	**0.000128**	**0.0321**	

Table 5: Tukey's test applied for the Elongation of MF with different Bloom indexes.

Bloom	B90 1.66	B250 24.3	B280 21.2
B90		**0.000128**	**0.000128**
B250	**0.000128**		0.315
B280	**0.000128**	0.315	

Figure 6 shows the results where the brittle behavior of the GeB90 fibrous membrane contrasts with the plastic behavior of GeB250 and GeB280. The elongation of GeB250 and GeB280 electrospun membranes was, on average, at least ten times higher than that of GeB90 membranes. The increase is in agreement with a greater extensibility of the films containing greater amounts of triple-helix structure, which is proportional to the Bloom index (Bigi *et al*. 2004). In other words, by increasing molecular weight, a greater number of molecules seem to hold together the polymeric network. This causes an increase in flexibility and, consequently, higher tenacity.





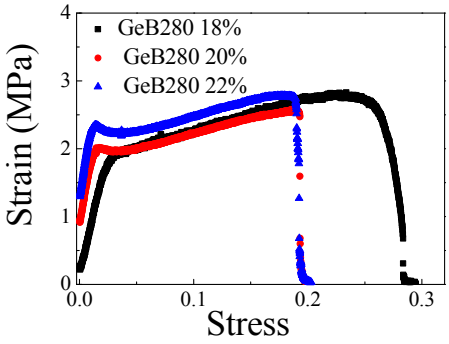


Figure 6: Stress-strain curves of electrospun GeB90, GeB250 and GeB280 at different concentrations.

CONCLUSIONS

In this work, the spinnability of gelatin solutions and the corresponding mechanical properties of the fibrous membranes formed were investigated. Taking into account the fact that viscosity is the most important property for obtaining a successful electrospinning, the lower the Bloom index of the gelatin, the more concentrated the solution should be to obtain ideal viscosity. Results indicated that an adequate viscosity range to obtain a homogeneous fibrous membrane is above 300 and below 700 mPa.s. Hence, high Bloom gelatin can be a more economical alternative than low Bloom gelatin. The fibrous membranes electrospun from GeB90 showed different mechanical behavior than GeB250 and GeB280, suggesting that low Bloom gelatin produces brittle fibrous membranes, while high Bloom gelatin generates membranes exhibiting plastic behavior.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of CNPq. Research supported by LASIPO, Central of Analysis/EQA and LCME, Federal University of Santa Catarina - UFSC.

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