

Structure and Physical Properties of Gelatin Fibers Prepared by Gel-Spinning in Ethylene Glycol

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ABSTRACT: Gelatin fibers were produced by gel-spinning using ethylene glycol as the solvent. Gelation conditions, drawing temperature, and draw ratio were studied. Physical properties of the fibers were characterized by mechanical tests, thermal analysis, X-ray diffractometry, and scanning electron microscopy. The mechanical strength of the fibers increased with increased draw ratio. The gelatin fiber prepared by extrusion of the gelatin (15 wt %)/ethylene glycol into methanol at -20°C and drawn up to seven times its original length at 5°C had the highest tensile

strength (405 MPa), a Young's modulus of 11 GPa, and a storage modulus of 7.9 GPa at 200°C . Remarkable differences were observed between the morphology of the undrawn and the drawn fibers. The results show that a gelatin fiber with very good mechanical properties can be prepared using ethylene glycol as the dispersion medium. © 2012 Wiley Periodicals, Inc. *J Appl Polym Sci* 000: 000–000, 2012

Key words: gelatin; fibers; gel spinning; ethylene glycol; mechanical properties

INTRODUCTION

Gelatin, a macromolecule obtained by partial hydrolysis of collagen, has widespread applications in the pharmaceutical and related medical fields, including sealants for vascular prostheses, wound dressings, and drug delivery.^{1–3} Gelatin is also widely used in the food and cosmetics industries, as well as in the preparation of photographic emulsions.^{4,5} Some applications of gelatin are attributed to its excellent biodegradability and biocompatibility, as well as the ease of modulating its physicochemical properties suitably.

Producing gelatin in the form of fibers and sponges enhances its industrial applications, particularly in the field of regenerative medicine where biocompatibility is crucial.⁶ In general, collagen-based biomaterials such as gelatin fibers (GF) often need reinforcement to preserve their structure during the course of specific applications.⁷ In view of this, physical and chemical methods are often used to provide structural stability. The physical methods include ultraviolet and gamma irradiation, and plasma treatment as well as hydrothermal conditioning.^{8–10} The chemical

methods often involve the use of crosslinkers in the form of bisfunctional and polyfunctional reagents, such as formaldehyde, glutaraldehyde, diisocyanates, and polyepoxy compounds, which make use of the large number of functional side groups on gelatin for the crosslinking reaction.^{11–14}

In earlier work, a gelatin fiber was prepared by a wet-spinning process using a solution of gelatin in LiCl-*N,N*-dimethylacetamide containing glutaraldehyde and a concentrated aqueous solution of gelatin with a crosslinking reagent.¹⁵ In another report, a gelatin fiber was prepared by wet spinning a 20-wt % aqueous solution of gelatin into methanol.¹⁶ The chemical crosslinking methods are simple and effective in the production of GF; however, several crosslinking reagents, e.g., glutaraldehyde, are cytotoxic and chemical crosslinking is often detrimental to the biocompatibility and biodegradation inherent in gelatin.^{12,17} Furthermore, the chemical crosslinking of fiber molecules makes the orientation of the chain along the fiber axis difficult, thus limiting the mechanical strength of such fibers.¹⁸ Producing strong GF without the use of a crosslinking reagent would facilitate the production of pure fibers for medical use and other applications.

Earlier studies confirmed that the mechanical strength of a gelatin film prepared by casting an aqueous solution of gelatin can be improved remarkably by drawing.^{19,20} Tanioka et al. reported that drawing a gelatin film in a mixture of water and ethanol results in reconstitution of the triple helix

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collagen-fold structure.²¹ Drawing a spun fiber in the gel state could enable the preparation of GF with the desired mechanical properties owing to reconstitution of the triple helix collagen-fold structure and its orientation.

In an earlier study, we showed that gelatin fiber in the gel state without chemical crosslinking reagents has a high drawability and orientation of the pseudocrystallite along the fiber axis.²² Furthermore, we have reported the mechanical properties of GF prepared by a gel-spinning method using dimethyl sulfoxide (DMSO) as the solvent.²³ Despite not using any crosslinking reagent, the fiber produced from the process exhibited a tensile strength (σ_b) of 146 MPa and a Young's modulus (E) of 2.3 GPa when drawn to the maximum length. This report indicated that the choice of the solvent used in the spinning process influences the mechanical properties of the resulting gelatin fiber and that using ethylene glycol as a solvent is effective in improving the properties of the fiber. Therefore, the present study was designed to investigate the conditions of preparing GF from a gelatin/ethylene glycol solution in a gel-spinning process with special attention to the gelation temperature, drawing temperature, and draw ratio, which is the ratio of the length of the drawn fiber to its original length. In addition, mechanical properties were studied with a view to providing relevant information for the structural characterization and practical applications of the GF produced.

EXPERIMENTAL

Material

Gelatin (Type A, ~300 bloom grade) prepared from porcine skin was supplied by Sigma-Aldrich. Ethylene glycol (99.5%) and methanol (99%) were purchased from Wako Pure Chemical Industries, Osaka, Japan, and used without further purification.

Fiber preparation

Gelatin (15 wt %) in ethylene glycol was heated at 85°C in an oil bath with gentle stirring and extruded at 0.072 mL/min through a nozzle (0.5-mm internal diameter and 15 mm height) at 48°C into a temperature-controlled bath of methanol. The air gap between the nozzle tip and the bath surface was 10 mm, and the gel fiber was pulled for 1 min through methanol in a bath set at either -10 or -20°C.

The gelled fibers were wound directly onto a bobbin and drawn at different draw ratios in the case of the drawing temperature equal to the gelation temperature. In the other cases, after the gelled fibers were wound onto a bobbin, they were kept

for 10 min at 5°C or at room temperature ($30 \pm 2^\circ\text{C}$) before drawing. The drawing of the fibers was done to different degrees of elongation by rolling them on two motor-driven winders set at different speeds. The drawn fibers were then immersed in methanol at room temperature for 10 days to extract the ethylene glycol while keeping the fiber at the length obtained immediately after drawing and then dried at room temperature for 24 h.²²

Measurements

Tensile tests were measured on single fibers of 20-mm length each at a crosshead speed of 20 mm/min using a Tensilon Universal Tester RTC-1350A (Orientec). The cross-sectional area of a fiber specimen was measured under a light microscope with an Objective micrometer (Olympus). The tensile strength and Young's modulus were evaluated from stress-strain curves, and the data are presented as averages from the measurement of >20 samples.

Wide-angle X-ray diffraction patterns were recorded on an imaging plate for 30 min with an X-ray diffractometer (RAXIS-IV; Rigaku), operated at 50 kV and 100 mA with a graphite-monochromated $\text{CuK}\alpha$. Scanning electron micrographs were taken with a JCM-5000 NeoScope (JEOL). Samples were sputtered with gold using a Neocoater MP-19010 NCTR before observation.

The dynamic storage modulus and the loss modulus were measured on a 20 mm length of a single fiber at a heating rate of 2°C/min using a dynamic viscoelastic analyzer (Rheo-Station DVE-V4; UBM). The test was strain controlled at an amplitude of 4 μm and at a frequency of 110 Hz.

Birefringence (Δn) was determined from eq. (1) using an Olympus BH-2 polarizing microscope equipped with a Berek compensator:

$$\Delta n = (c/10^4) \times 10^4 f(i)/d \quad (1)$$

where $i = (a + b)/2$, c is the constant (in nm) of the compensator. a is the graduation of the compensator when the top of the zero-order stripe corresponds to the cross-point of the polarizing microscope, b is the graduation in the reverse direction, $f(i)$ is a constant determined by the i value, and d is the diameter (in μm) of the fiber.²⁴ Samples were soaked in a 1:1 (v/v) mixture of di- n -butyl phthalate and tricresyl phosphate.

Differential scanning calorimetry (DSC) with a Thermoflex DSC 8230 (Rigaku) was done at a heating rate of 5°C/min under a nitrogen atmosphere. Approximately 0.5 mg of each fiber cut into pieces <1 mm length was sealed in an aluminum pan for the measurement.

TABLE I
Conditions of Preparation of Gelatin Fibers

Sample	Temperature of gelation (°C)	Temperature of drawing (°C)
GF1	-20	-20
GF2	-20	5
GF3	-20	RT ^a
GF4	-10	-10
GF5	-10	RT ^a

^a Room temperature ($30 \pm 2^\circ\text{C}$).

RESULTS AND DISCUSSION

Mechanical properties

The conditions of gelation and drawing and the obtained GF are listed in Table I. A gelatin (15 wt %)/ethylene glycol solution did not yield a spun fiber with sufficient flexibility to wind onto a bobbin when extruded into a methanol bath held below -20°C , and it did not become gel at temperature above -10°C . At 5°C , the spun fiber gelated at -20°C could be drawn maximally to seven times its original length. Figure 1 shows typical stress–strain curves recorded for the GF2 fibers from which the dispersion medium has been extracted completely after drawing. The tensile strength of the undrawn fiber was low and was increased by drawing.

The tensile strength and the Young's modulus evaluated from the stress–strain curves of fibers prepared under various conditions are presented in Figure 2. The GF2 fiber drawn to maximum ratio had a σ_b value of 405 MPa and an E value of 11.4 GPa. The GF3 fiber, which was prepared at a gelation temperature of -20°C and drawn at room temperature, also could be drawn up to seven times its original length, where it had a σ_b value of 297 MPa and an E value of 8.9 GPa. The strength of the GF1 fiber prepared at both gelation and drawing temperatures of -20°C was less than

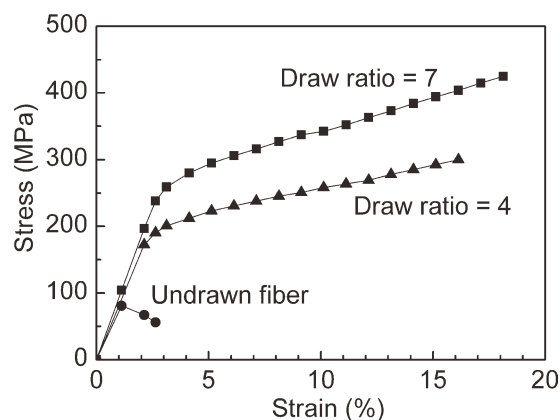


Figure 1 Typical stress–strain curves recorded for undrawn and drawn GF2 fibers.

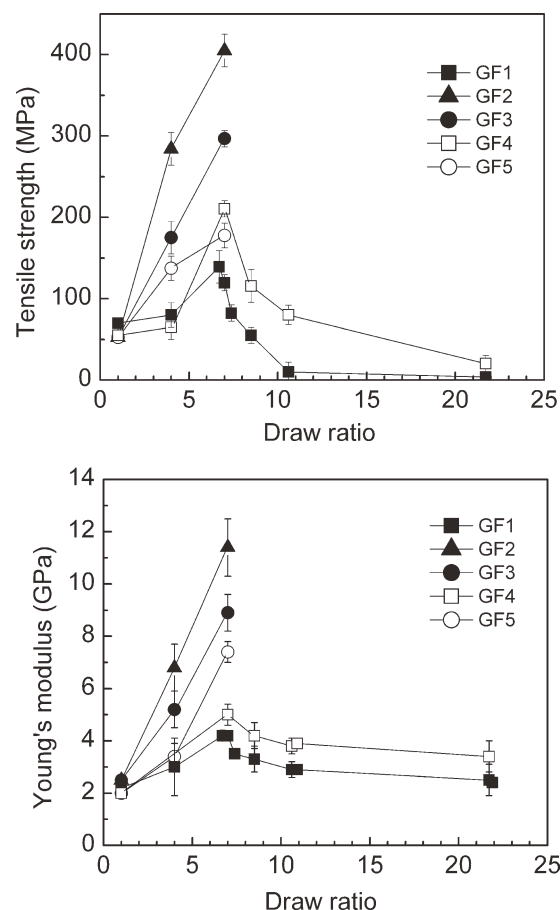


Figure 2 The tensile strength and the Young's modulus of gelatin fibers prepared under different conditions.

that of GF3. The drawing temperature is important in the preparation of fibers as it affects the orientation along the fiber axis. A low degree of orientation of the fiber molecular chains occurs when the drawing temperature is too low because the molecular chains in the gel fibers cannot move freely to rearrange, whereas the movements of the molecular chains is very active to maintain the arrangement along the fiber axis when the drawing temperature is high.

Though GF1 and GF4 fibers, which were prepared at the drawing temperature equal to the gelation temperature, respectively, could be drawn up to 22 times their original length, the strengths of the fibers did not increase monotonously with the increase in the draw ratio; rather, the strength of these fibers decreased at draw ratios of more than seven times its original length. Stretching beyond seven times the original length probably caused a crash, which could be due to the longitudinal splitting of the fibrils along the fiber axis, thereby limiting the mechanical properties of the fiber. It appears that drawing to approximately seven times the original length is suitable to improve the strength of spungel fibers under the conditions used in this study;

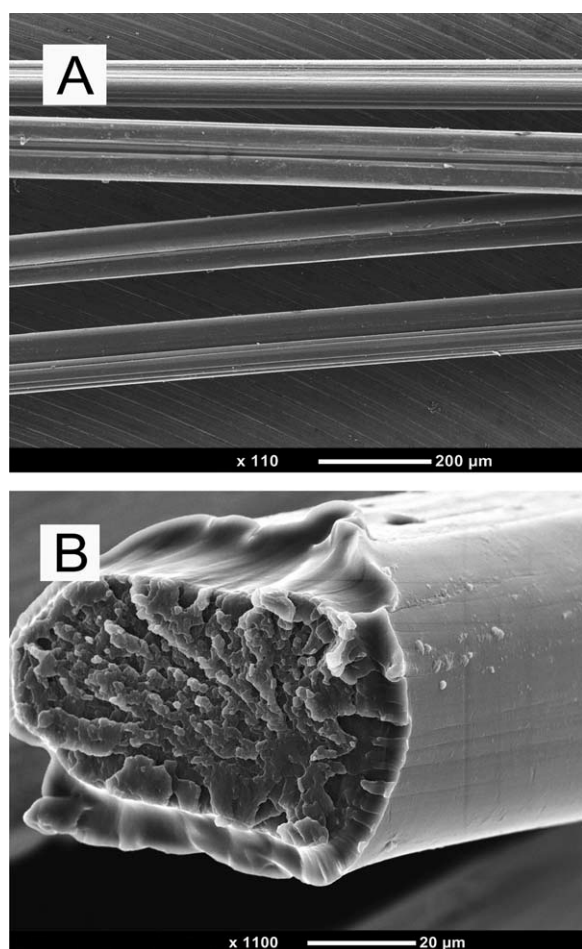


Figure 3 The scanning electron micrographs of a GF2 fiber drawn to seven times its original length. (A) Side view, magnification 110 \times ; (B) cross section, magnification 1100 \times .

i.e., the molecular weight of gelatin, the concentration, and the temperature of the spinning solution.

Earlier, we reported the preparation of gelatin fiber using DMSO as the solvent.²³ In that study, a fiber with the optimum mechanical property was prepared at a gelation temperature of -20°C , a draw temperature of 20°C and a maximum draw ratio of 16. The fiber had a σ_b value of 146 MPa and an E value of 2.3 GPa. The fiber prepared by using ethylene glycol as the spinning solvent showed a lower degree of drawability compared to those prepared by using DMSO as the spinning solvent but the tensile strength and Young's modulus were higher.

Drawing gelatin fiber enhances the renaturation of random gelatin strands to the triple-helix structure that exists in native collagen.²⁵ In addition, Tanioka et al. reported that the development of a triple-helix structure in gelatin increases with increasing deformation.²¹ We showed in earlier work that effective drawing in a gel state enhances arrangement of the molecular chains and formation of a triple-helix structure, resulting in improved fiber strength.²²

Ethylene glycol probably has greater affinity with gelatin molecules, which results in enhanced cleavage of the association of gelatin molecules allowing their rearrangement.²⁶

Scanning electron microscopy and birefringence

Scanning electron micrographs of a GF2 fiber prepared at a gelation temperature of -20°C and drawn to seven times its original length at 5°C and its cross section is shown in Figure 3. Skin and core sections were clearly observed in the fibers produced by gel spinning. The development of the skin is attributed to the deposition of gelatin molecules on the surface of the fiber as ethylene glycol, the dispersion medium of the gel, diffuses into methanol in the cooling bath. Longitudinal section micrographs of the undrawn and the drawn fibers are shown in Figure 4. The micrograph of the undrawn fibers showed that the formation of fibrils in the gel state was

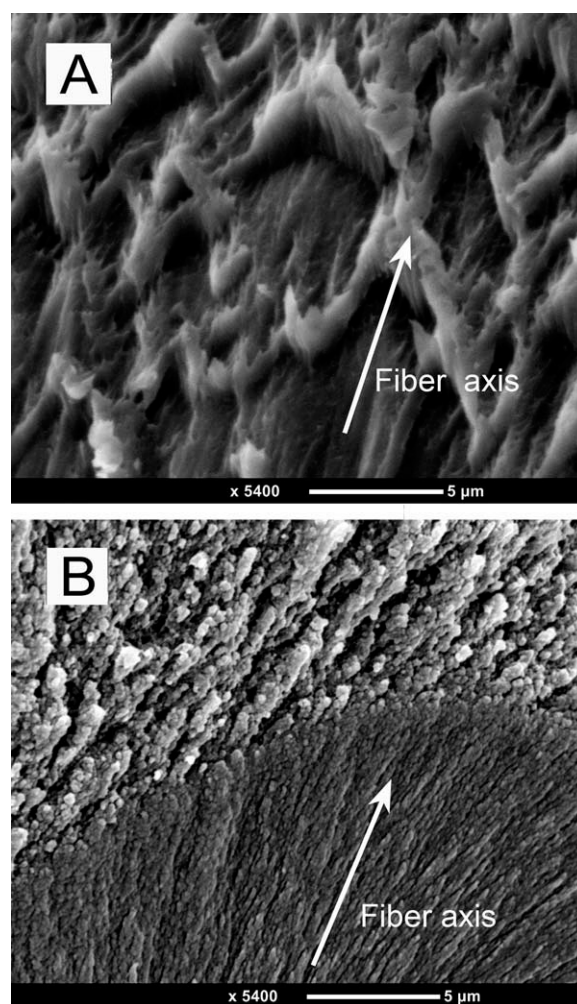


Figure 4 Scanning electron micrographs of the longitudinal section of a GF2 fiber (magnification 5400 \times). (A) Undrawn fiber; (B) fiber drawn to seven times its original length.

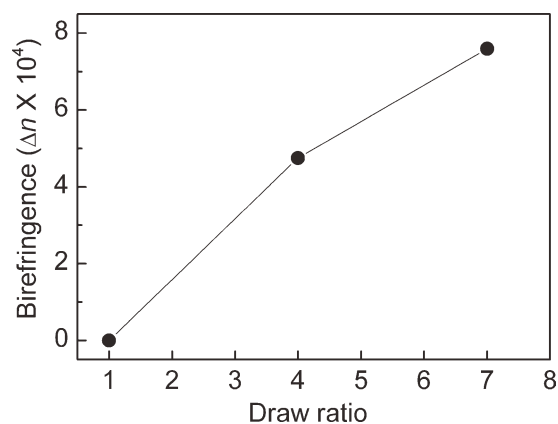


Figure 5 Change in the birefringence of GF2 fiber with draw ratio.

incomplete and the few fibrils that did develop were randomly distributed. The alignment of the fibrils along the direction of deformation was observed in the drawn fiber. This alignment suggests that the chain segments of gelatin molecules are orientated along the fiber axis, which indicates improved mechanical properties.¹⁹ These results corroborate the findings on the mechanical properties as presented in Figures 1 and 2.

Figure 5 shows the change in the birefringence of GF2 with changing draw ratio. Increases in birefringence with increased draw ratio are clearly shown, indicating that anisotropy increases as the draw ratio increases. The anisotropy of a fiber polymer increases as the orientation of the molecular chains along the fiber axis increases, which is responsible for increased fiber strength.

X-Ray structure analysis

The X-ray diffraction patterns of GF2 fibers are presented in Figure 6. The fiber drawn to seven times its original length showed a clearer diffraction on the second layer-line and a sharper diffraction on the meridian than the undrawn fiber. In addition,

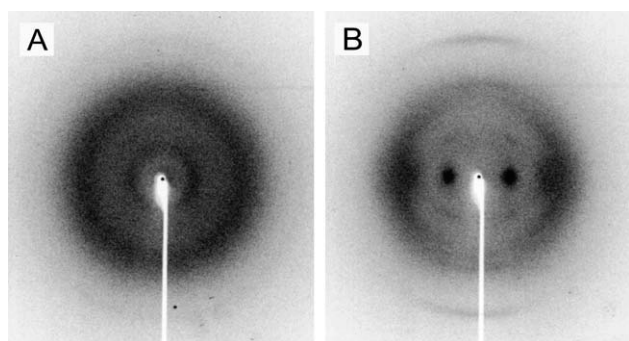


Figure 6 X-Ray diffraction patterns for GF2 fibers. (A) Undrawn fiber; (B) fiber drawn to seven times its original length.

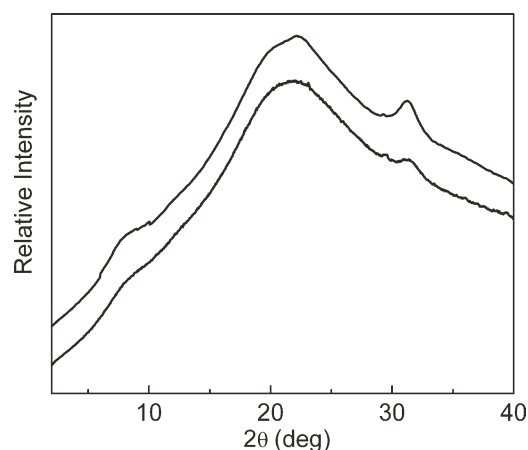


Figure 7 The X-ray diffraction profiles for GF2 fibers. Upper curve, fiber drawn to seven times its original length. Lower curve, undrawn fiber.

the undrawn fiber showed an isotropic pattern, whereas the drawn fiber showed an anisotropic pattern as the lattice spots were located near the equatorial axial line. These observations imply that the drawn fiber has a more nearly faultless crystal structure and a higher orientation of the fiber filaments along the fiber axis than the undrawn fiber.

The X-ray diffraction profiles of the GF are presented in Figure 7. The peak at $2\theta = 31^\circ$ is attributed to the diffraction arising from the repeat of amino acid residues involved in a triple-helix structure of collagen, whereas the peak at $2\theta = 8^\circ$ is assigned to the development of lateral bonding among the triple helices of the fibrils.²⁷ The intensities of the peaks at $2\theta = 31.3^\circ$ and $2\theta = 7.9^\circ$ are higher in the drawn fiber than those of the undrawn fiber. The ratio of the peak area of the former to the latter is 2.7 for the peak centered on 31.3° and 2.6 for the peak centered on 7.9° . Drawing a gelatin fiber probably facilitates the realignment of the triple helices and the

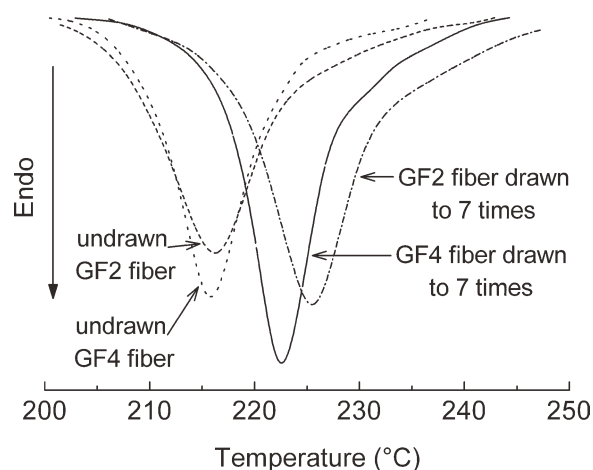


Figure 8 DSC thermograms of gelatin fibers prepared under different conditions.

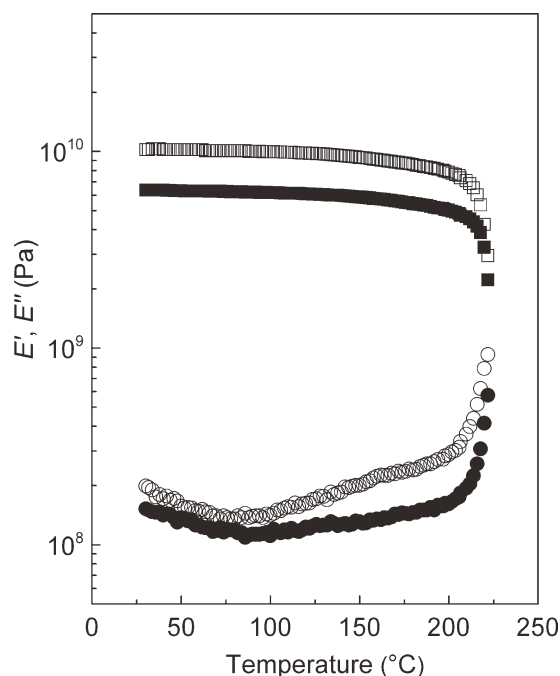


Figure 9 Temperature dependence of the dynamic storage modulus (E') and the loss modulus (E'') for GF2 fibers. (■ E' , ● E''), undrawn fiber; (□ E' , ○ E''), fiber drawn to seven times its original length.

development of crystallites in the drawn fiber. The intensities of the peaks correspond to the amount of crystallites. Similar observations have been reported for gelatin films prepared in water and ethanol.²¹

Thermal properties

DSC measurements were used to characterize the thermal properties of the fibers (Fig. 8). The fibers (GF2 and GF4) drawn to seven times their original lengths had higher melting points than the undrawn fibers. The amount of triple-helix structure in gelatin has been reported to increase with increased stretching.¹⁹ In addition, stretching the fibers enhances the development of pseudocrystallite owing to the associations among the triple helices. Therefore, the triple helices would require more heat to dissociate the intermolecular bonds among them into the molten form. This explains why the melting points of the drawn fibers are higher than those of the undrawn fibers. The higher melting point obtained in the drawn fibers means greater thermal stability, which may be harnessed for relevant applications.

Dynamic viscoelastic behavior

The temperature dependence of the dynamic storage modulus (E') and the loss modulus (E'') of the drawn and the undrawn GF2 fibers are shown in Figure 9. The storage moduli of the undrawn and

the drawn fibers at 30°C were 6.4 and 10 GPa, respectively. The higher E' observed in the drawn fiber is likely to be due to the higher orientation of the gelatin chain and the formation of large amounts of the triple helices, which act as pseudocrystallites. E'' of the drawn fiber was also higher than that of the undrawn fiber within the temperature range measured. E' of both the undrawn and the drawn fibers decreased at around 150°C; however, the drawn fiber maintained a value of 7.9 GPa at 200°C. This means that the drawn fiber has better mechanical strength and thermal stability for practical applications than the undrawn fiber. Earlier, we studied the dynamic viscoelastic behavior of a gelatin fiber prepared with DMSO for dispersion of the gel.²² In that report, E' of the fiber was 3 GPa at room temperature, which is fairly low compared to that of the fiber obtained in this study. Therefore, this study confirms that the use of ethylene glycol in the gel-spinning process for the preparation of gelatin fiber results in better mechanical properties and greater thermal stability.

CONCLUSIONS

Gelation temperature, drawing temperature, and draw ratios influence the mechanical properties of GF prepared with ethylene glycol as the solvent in the gel-spinning process. In this work, our findings reveal that the optimum conditions for the preparation of GF with gelatin/ethylene glycol solution is by spinning into methanol at a gelation temperature of -20°C and drawing at 5°C up to seven times its original length. Studies reveal that mechanical properties increased as the draw ratio increased but drawing the fiber beyond seven times its original length impaired the strength of the material. In addition, the fibers showed higher mechanical properties than similar fibers prepared using DMSO in our earlier work. The improvement of the mechanical properties of the fibers produced in ethylene glycol is probably due to a higher affinity between the solvent molecules and the gelatin molecules resulting in a more nearly perfect cleavage of the association of gelatin molecules for their rearrangement. Useful information on the gel-spinning preparation of gelatin fiber using gelatin/ethylene glycol solution has been provided by this work. The GF obtained in this study have mechanical and thermal properties comparable with those of commercial rayon staple. Therefore, they are expected to be useful in the development of new biocompatible materials; e.g., extracellular matrices for tissue engineering and new biodegradable materials through the production of both woven and nonwoven fabric.

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