Materials Science and Engineering C 63 (2016) 10–17

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Rheological properties of glutaraldehyde-crosslinked collagen solutions analyzed quantitatively using mechanical models

Zhenhua Tian [a,c], Lian Duan [a,b], Lei Wu [a], Lirui Shen [c], Guoying Li [a,c,*]

[a] The Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University, Chengdu 610065, PR China
[b] College of Textile & Garments, Southwest University, Chongqing 400715, PR China
[c] National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu 610065, PR China

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form 19 January 2016
Accepted 17 February 2016
Available online 18 February 2016

Keywords:
Collagen
Glutaraldehyde
Rheological properties
Mechanical models

ABSTRACT

Understanding the rheological behavior of collagen solutions crosslinked by various amounts of glutaraldehyde (GTA) [GTA/collagen (w/w) = 0–0.1] is fundamental either to design optimized products or to ensure stable flow. Under steady shear, all the samples exhibited pseudoplasticity with shear-thinning behavior, and the flow curves were well described by Ostwald-de Waele model and Carreau model. With increased amounts of GTA, the viscosity increased from 6.15 to 168.54 Pa·s at 0.1 s^{-1}, and the pseudoplasticity strengthened (the flow index decreased from 0.549 to 0.117). Additionally, hysteresis loops were evaluated to analyze the thixotropy of the native and crosslinked collagen solutions, and indicated that stronger thixotropic behavior was associated with higher amount of GTA. Furthermore, the values of apparent yield stress were negative, and a flow index <1 for all the systems obtained via Herschel–Bulkley model confirmed that the native and crosslinked collagen solutions belonged to pseudoplastic fluid without apparent yield stress. However, the increment of dynamic denaturation temperature determined by dynamic temperature sweep was not obvious. The viscoelastic properties were examined based on creep–recovery measurements and then simulated using Burger model and a semi-empirical model. The increase in the proportion of recoverable compliance (instantaneous and retardant compliance) reflected that the crosslinked collagen solutions were more resistant to the deformation and exhibited more elastic behavior than the native collagen solution, accompanied by the fact that the compliance value decreased from 39.317 to 0.152 Pa^{-1} and the recovery percentage increased from 1.128% to 87.604%. These data indicated that adjusting the amount of GTA could be a suitable mean for manipulating mechanical properties of collagen-based biomaterials.

1. Introduction

Collagen, the major structural protein of vertebrates and many other multicellular organisms, and its derived matrices have been used in many fields such as medicine, food, cosmetics and the chemical industry due to its low antigenicity, high biocompatibility, controlled biodegradability and good bioresorbability [1,2]. Although collagen is recognized as a promising material, concerns remain about the low mechanical strength, weak thermal stability and vulnerability to enzymatic degradation of native collagen. Generally, in order to meet the demands of *in vivo* and *in vitro* applications, collagen-based biomaterials require some chemical treatment. According to numerous studies [3–5], GTA was the most effective crosslinking agent to stabilize collagen against thermal and enzymatic degradation because of its low cost, high reactivity and high solubility in aqueous solution. Moreover, bioprosthesis valves and grafts treated with GTA have been implanted in thousands of patients over the last several decades and the clinical outcomes indicate that GTA is clinically acceptable in spite of the reports on its cytotoxicity [6].

In most cases, collagen is exposed to a certain strain/stress during the machining process for the preparation and molding of the collagen-based biomaterials and its mechanical properties such as viscidity, elasticity and deformation are affected by the shear and extrusion. Therefore, it is necessary to investigate the mechanical properties of the GTA-crosslinked collagen in order to optimize the design and fabrication of collagen-based biomaterials. For example, Friess and Schlapp [7] investigated the effect of chemical crosslinking on the rheological properties of insoluble collagen fibril dispersions by reacting it with GTA, and then found that GTA led to increases in both the viscosity and resistance to deformation of the collagen dispersions. Moreover, the collagen gel solutions were prepared by reacting collagen solution (10 mg/mL) with various concentrations of GTA, and the viscoelasticity was measured using dynamic mechanical analysis [8]. The results showed that the elastic modulus, viscosity and retardation time of the collagen gel solutions increased, indicating a gradual transformation from liquid behaviors to solid characteristics as the GTA concentration

* Corresponding author at: The Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University, Chengdu 610065, PR China.
E-mail address: liguoyings@163.com (G. Li).

http://dx.doi.org/10.1016/j.msec.2016.02.047
0928-4931/

increased. In comparison with the GTA-crosslinked collagen fibril and gel solutions, the crosslinked collagen solution is more homogeneous. Moreover, biomedical collagen preparations are mainly based on aqueous solution preparations either used directly as injectable [9] or molded into gels or solids (such as sponges, films, tubes and powder) for tissue regeneration or drug delivery [1,10,11]. Consequently, the preparation of uniform GTA-crosslinked collagen solutions and the study of their rheological properties are of considerable important. Nevertheless, to date, few studies have focused on the rheological properties of the GTA-crosslinked collagen solutions.

In the authors' previous work [12], collagen solutions (5 mg/mL) with various amounts of GTA [GTA/collagen (w/w) = 0–0.5] were prepared, and the crosslinking degree, dynamic viscoelasticity, thermal stability and morphological characteristics were examined. At low GTA amounts [GTA/collagen (w/w) ≤ 0.1], the crosslinking degree increased largely from 0 to 67.24%, accompanied by obvious increases in the fiber diameter and the values of G', G'' and η^*. However, the thermal denaturation temperature (T_d) improved slightly and the fluidity of collagen samples was still retained. When the ratio of GTA to collagen exceeded 0.1, although the crosslinking degree only increased by ~8.2%, the crosslinked collagen solution displayed a sudden rise of the T_d value and a clear loss of flow. Additionally, the G' and G'' values were nearly constant within the frequencies tested, suggesting that the samples exhibited typical gelatinous characteristics and were mechanically stable under dynamic oscillation. These results indicated that the crosslinked collagen solutions were homogeneous and could hold good fluidity at the GTA/collagen (w/w) ratio ≤ 0.1; however, the collagen solutions were converted into gels when the GTA/collagen (w/w) ratios >0.1. Moreover, the semi-solids (collagen gel) would rupture at high shear rate, resulting in an interstice and providing meaningless data [13]; therefore, the steady shear measurement and thixotropic measurement are unsuitable for the crosslinked collagen solutions when the GTA/collagen ratios >0.1.

Consequently, the appropriate GTA/collagen (w/w) ratios were set from 0 to 0.1 in the present work to prepare the uniform GTA-crosslinked collagen solutions with good fluidity. Then the rheological properties (steady shear measurement, thixotropy, dynamical temperature sweep and creep–recovery measurement) were measured using a rotational rheometer and an attempt was made to simulate and quantitatively analyze the experimental data using mechanical models.

2. Materials and methods

2.1. Extraction of the calf skin collagen

Collagen was prepared from calf skin using acetic acid containing 1% pepsin (EC 3.4.23.1, 1:10,000, Sigma Chemical Co.) according to the method of Zhang et al. [14]. The supernatant of extracted mixture was collected by centrifugation (10,000 × *g*, 10 min) at 4 °C and salted out in 3 mol/L NaCl solution. Then the precipitated collagen was dissolved in 0.5 mol/L acetic acid and salted out by adding NaCl to a final concentration of 0.7 mol/L. The precipitate was again dissolved in 0.5 mol/L acetic acid, and then dialyzed against 0.1 mol/L acetic acid for 3 days to remove NaCl. The purity and molecular weight of the obtained collagen were judged by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE pattern of the sample displayed two α bands (~100 kDa for α1 and α2) and one β band (~200 kDa) which were typical electrophoretic bands of type I collagen, indicating the collagen with high purity preserved original triple helix and its molecular weight was about 300 kDa [14,15]. Finally, the collagen solution was lyophilized with a freeze dryer (Labconco Freeze Dryer FreeZone 6 Liter, USA) at −50 °C for about 2 days and then stored at 4 °C for no more than 3 months. All other reagents were analytical reagent grade and used without further purification.

2.2. Preparation of the crosslinked collagen solutions

The crosslinked collagen solutions were prepared using the method of Tian et al. [12] with some modification. Lyophilized collagen was dissolved in 0.2 mol/L acetic acid–sodium acetate buffer solution (pH 4.00) to obtain 5 mg/mL collagen solution and GTA (50%) was diluted to 5% with 0.2 mol/L acetate buffer solution (pH 4.00). Then, 5% GTA solution was added dropwise to collagen solutions, and the final GTA/collagen (w/w) ratios were 0:1, 0.01:1, 0.03:1, 0.05:1, and 0.1:1. These collagen samples were named as COL (native collagen), GC1, GC2, GC3, and GC4, respectively. After these solutions were stirred incessantly for 24 h at room temperature (~20 °C), excess glycine was added into the mixed solutions to react with the residual GTA. The resultant collagen solutions were centrifuged at 8000 × *g* for 10 min to remove entrapped air-bubbles, and then stored at 4 °C until used.

2.3. Steady shear measurements

The rheological properties of the native and crosslinked collagen solutions were measured using a rotational rheometer (Haake Mars III, Germany) equipped with stainless steel parallel plate geometry (35 mm diameter and 2 mm gap). The temperature was controlled using a temperature controller with an accuracy of ±0.1 °C.

The steady shear test was performed in the controlled rate (CR) mode at 25 °C by increasing the shear rate from 0.05 to 100 s^{-1}. The obtained flow curves were fitted via the Ostwald-de Waele model (Eq. (1)) and Carreau model (Eq. (3)), which were successfully used to further analyze the relationship between shear viscosity and shear rate of systems related to our samples [16,17]:

$$\eta = K\dot{\gamma}^{n-1} \tag{1}$$

where η is the shear viscosity (Pa·s), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency coefficient (Pa·s^{n-1}) and n is the flow index (dimensionless). The value of n is not equal to 1 for non-Newtonian fluids and it determines the type of fluid category it belongs to. Value of parameter $n < 1$ represents the sample is a pseudoplastic fluid, whereas value of $n > 1$ indicates a dilatant fluid [18,19]. The more "n" deviates from "1", the stronger the non-Newtonianism of sample is:

$$(\eta-\eta_\infty)/(\eta_0-\eta_\infty) = \left[1+(\lambda\dot{\gamma})^2\right]^{\frac{m-1}{2}} \tag{2}$$

where η_0 is the zero-shear viscosity (Pa·s), the constant viscosity in the first Newtonian plateau ($\dot{\gamma}\to 0$), η_∞ is the infinite viscosity (Pa·s), the constant viscosity in the second Newtonian plateau ($\dot{\gamma}\to\infty$), λ is a characteristic time (s) and m is a dimensionless constant corresponding to the flow index n [20,21].

Because the second Newtonian region was not approached in our experiments, and the parameter η_∞ is usually ignored as a result of it being small relative to η and η_0 ($\eta \gg \eta_\infty$ and $\eta_0 \gg \eta_\infty$), it could be assumed that $\eta - \eta_\infty \approx \eta$ and $\eta_0 - \eta_\infty \approx \eta_0$; therefore, Eq. (2) was simplified to comprise only three parameters [22]:

$$\eta = \eta_0\left[1+(\lambda\dot{\gamma})^2\right]^{\frac{m-1}{2}}. \tag{3}$$

Additionally, the regression coefficient (R^2) and standard deviation (SD) were used as indicators of the accuracy.

2.4. Thixotropic measurements

The thixotropic curves for the native and crosslinked collagen solutions were examined under the CR mode at 25 °C. In the tests, the shear rate increased from an initial minimum of 0.01 s^{-1} to a maximum of 50 s^{-1} in 150 s firstly (up curve), and then decreased from 50 to