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ORIGINAL PAPER

Viscoelastic Properties of a Biological Hydrogel Produced from Soybean Oil

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Abstract Hydrogels formed from biopolymers or natural sources have special advantages because they may have biodegradable and biocompatible properties. The viscoelastic properties of a newly developed biological hydrogel made from epoxidized soybean oil (ESO) were investigated. The material called HPESO is a hydrolytic product of polymerized ESO (PESO). HPESO exhibited viscoelastic solid or gel behavior above 2% (wt. %) concentration at room temperature and viscous liquid behavior at 55 °C. The thermal assembly disassembly reassembly function of the HPESO hydrogel was completely reversible. The viscoelastic properties of HPESO were dependent on concentration. Analysis of modulus and concentration dependence and stress relaxation measurement indicated that HPESO was a physical gel where the cross-linkers between the molecules were physical junctions. HPESO hydrogel also exhibited fast initial recovery of its viscoelastic properties after being subjected to mechanical shear disruption. The function and behavior of the HPESO hydrogel suggest that this biomaterial may be suitable for applications in drug delivery and scaffolds of bioengineering and tissue engineering.

Keywords Biodegradable material · Hydrogel · Rheology · Soybean oil · Viscoelastic properties

Introduction

Hydrogels are viscoelastic materials that have many biomedical utilization applications, such as drug delivery, wound care products, breast implant materials, tissue engineering, etc. [1–4]. Hydrogels can be made from synthetic polymers such as ethylene oxide and vinyl alcohol. Some biological hydrogels obtained from natural sources such as gelatin, fibrin, collagen, chitosan, etc. Biopolymers have potential advantages compared with synthetic polymers due to their biodegradable properties and, in many cases, lower cost. Hydrogels produced from biopolymers and/or natural sources have particular advantages in vivo applications since they are more likely biodegradable, biocompatible and bioresorbable.

Because soy-oil-based composites contain fatty acid residues that can be readily attacked by lipase-secreting bacteria, the family of the materials is generally regarded as biodegradable. Until the recent surge in biodiesel production, the United States annually produced about 0.45 billion kg (1.0 billion lb) of soybean oil in excess of commercial demand. This excess capacity resulted in lower prices for soybean oils as well as other agricultural commodities. New applications for these materials are being developed and exploited by polymer and biological scientists who are interested in these products [5].

Soybean oil is composed of triglycerides with fatty acids containing double bonds. By reacting with peroxides, soybean oil can be converted into epoxidized soybean oil (ESO). Wool et al. [6] reported that epoxy-containing soybean oil could be synthesized into new polymers suitable for liquid molding. Liu et al. [7] developed a new polymer using ESO. ESO was cross-linked into a polymer by thermal polymerization with triethylene glycol diamine (TGD). This biodegradable composite is a rubber-like material with the

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potential to replace synthetic rubber and plastics [5]. Recently, Liu et al. [8] developed a new biopolymer hydrogel using ESO. In order to further explore its usage and application, its rheological properties need to be examined. In this paper, the viscoelastic properties of hydrolytic polymerized ESO (HPESO) hydrogel were investigated.

Materials and Methods

Materials

Epoxidized soybean oil was obtained from Elf Atochem Inc. (Philadelphia, PA) and used as received. Boron trifluoride diethyl etherate, $(C_2H_5)_2\ O{\cdot}BF_3$, purified and redistilled, was provided by Aldrich Chemical Inc. (Milwaukee, WI, USA). Methylene chloride was purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Ring-Opening Polymerization of ESO (PESO) in Methylene Chloride

In a typical synthetic procedure, About 30 g of ESO and 300 ml methylene chloride were added to a 500-ml flask fitted with a mechanical stirrer, condenser, thermometer, nitrogen line and dropping funnel. The solution was cooled to 0 °C by using an ice bath. BF_3 (diethyl etherate), 0.396 g, was added drop-wise over 2 min. The system was maintained for 3 h. The methylene chloride was removed using a rotary evaporator and dried under vacuum at 70 °C to a constant weight and 29.8 g of polymer was obtained. The process was referred to as ring-opening polymerization of ESO (PESO), which will be reported in detail in another publication [8].

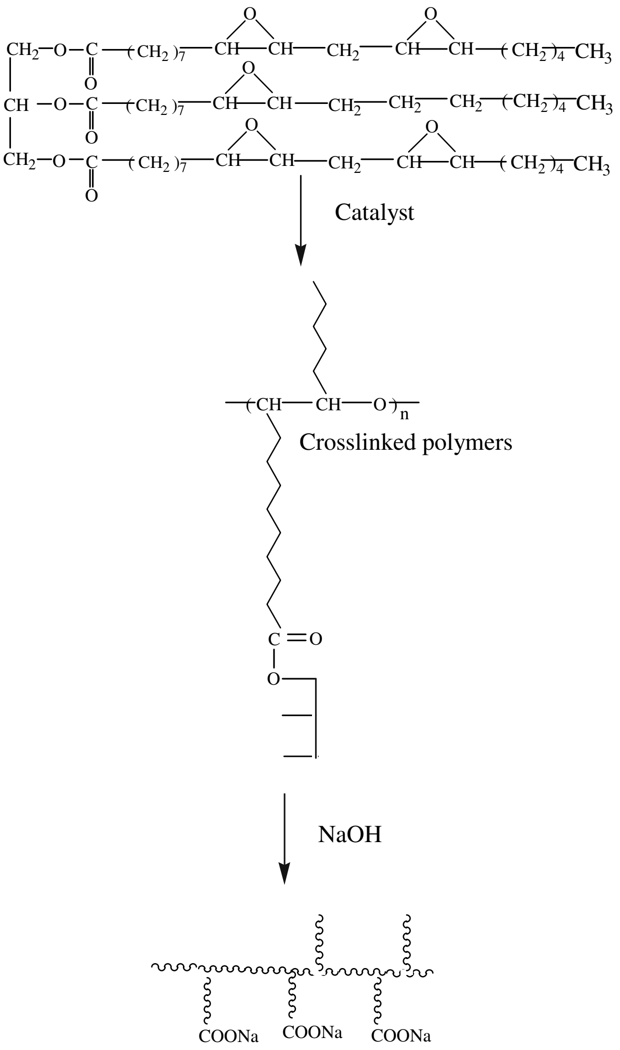
Hydrolysis of PESO by NaOH Solution

PESO, 2.5 g in 50 ml 0.4 N NaOH was refluxed for 24 h, and then the solution was filtered through filter paper and cooled to room temperature. The resulting gel was precipitated with 80 ml 1.0 N HCl, followed by washing several time with water and then twice with 10% (v/v) acetic acid. The resulting polymer was dried overnight in an oven at 80 °C and then dried under vacuum at 70 °C to constant weight. The product (2.1 g) was referred to as pre-HPESO.

Preparation of the Hydrogel

Pre-HPESO was fully neutralized by using a NaOH aqueous solution based on the ion-exchange value, and then diluted with phosphate buffer to the desired concentration and stirred with a magnetic bar for 6 h at 70 °C in a water bath. The neutralized pre-HPESO was called HPESO. When the HPESO solution was cooled to room temperature, a gel formed. When heating the gel to 55 °C, it became a solution again and was placed on the rheometer plate and rheological measurements were taken directly.

The structures and procedures stated above are shown as following:



Rheological Property Measurements

A strain-controlled Rheometric ARES rheometer (TA Instruments, New Castle, DE, USA) was used to determine the rheology properties. A 50-mm diameter cone-and-plate geometry was used. The cone angle was 0.04 radians. The temperature was controlled at 55 ± 0.1, 25 ± 0.1, or 37 ± 0.1 °C by using a water circulation system. A steady-shear experiment was performed only at 55 ± 0.1 °C. Linear viscoelastic measurements were conducted using the HPESO hydrogels. To ensure that all the measurements were made within the linear viscoelastic range, a strain-sweep experiment was conducted. An applied shear strain value in the linear range was adopted for the other viscoelastic property measurements; fresh samples were used for each experiment. Linear viscoelasticity indicated that the measured parameters were independent of shear strain. Time sweep experiments were conducted to monitor the gelation process over time. Small-amplitude oscillatory shear experiments were conducted over a frequency (ω) range of 0.1–500 rad/s, yielding shear storage (G') and loss (G'') moduli.

The storage modulus represents the non-dissipative component of mechanical properties. The elastic or "rubber-like" behavior is suggested if the G' spectrum is independent of frequency and greater than the loss modulus over a certain range of frequency. The loss modulus represents the dissipative component of the mechanical properties and is characteristic of viscous flow. The phase shift or phase angle (δ) is defined by $\delta = \tan^{-1}$ (G''/G'), and indicates whether a material is solid with perfect elasticity ($\delta = 0$), or liquid with pure viscosity ($\delta = 90°$), or something in between ($0 < \delta < 90°$).

Stress relaxation experiments measured the stress relaxation over time after the material was subjected to a step increase in shear strain. The plateau modulus was obtained using the method of Xu et al. [9]. The plateau modulus indicated that the stress relaxation measured modulus was nearly constant over the measured range of time.

Results and Discussion

The structure of ESO is similar to that of soybean oil, thus properties of ESO were similar to soybean oil. ESO behaved as a viscous Newtonian fluid [5]. HPESO hydrogel is a product of hydrolytic polymerized ESO (PESO). We investigated five concentrations of HPESO (1–5%, wt. %) at pH 8.0. At 55 °C, HPESO behaved as a perfect viscous liquid with viscosities close to water. No elasticity (G') could be detected at 55 °C. Its viscosity was about 1.1 cP and nearly independent of shear rate at 55 °C. This viscosity is very close to water with a slightly higher value due to the greater molecular weight of HPESO. At 55 °C, all five concentrations of HPESO exhibited the same behavior with little difference in viscosity values (data not shown) since the concentrations of HPESO used were relatively low. At 55 °C, HPESO displayed Newtonian fluid and viscous liquid behavior at the concentrations tested. At 25 °C, however, HPESO formed a gel after several hours of cooling from 55 °C to room temperature (Fig. 1a). Figure 1b shows micrographs of a 5% HPESO hydrogel and the micro-structure of the gel was pretty uniform.

In order to evaluate the rheological behavior of the HPESO hydrogel, a series of viscoelastic measurements were conducted. At first, the thermal dependence of gelation for HPESO was examined by cycling a HPESO gel through a series of test of dynamic temperature steps ranging from 55 to 25 °C at pH 8.0. Figure 2 shows the change of the elastic modulus (G') versus temperature for 3% HPESO. The solid curve in Fig. 2 shows the data obtained from 55 to 25 °C during the cooling portion of the cycle; the dashed line shows the heating from 25 °C back to 55 °C. Although the kinetics of the de-gelation and gelation were different due to the various rates of molecular association (assembly) and dissociation (disassembly), the thermal sol–gel procedure was completely reversible and repeatable for 2–5% HPESO (data not shown for samples of other concentrations). Because 1% HPESO was too dilute, it did not form a gel but exhibited weak viscoelastic behavior at 25 °C. The pH range for the HPESO to form a gel was between 7.4 and 14.

To monitor the gelation process, a time sweep measurement was conducted at 25 °C with 0.05% of strain amplitude and 1 rad/s frequency. At 25 °C, HPESO immediately exhibited viscoelastic behavior (Fig. 3), which implied that the molecules were cross-linked and associated. For 3 and 5% HPESO shown in Fig. 3, the storage or elastic modulus (G') jumped to 90 and 200 Pa, respectively, within a few seconds when measured at 25 °C and 1 rad/s frequency. A plateau of 1,200 Pa was reached after 3 h for 5% HPESO, and a plateau of 260 Pa was reached after 8 h for 3% HPESO (Fig. 3). The gelation kinetics strongly depended on concentration. We used a first-order association model to fit the kinetics of the gelation at 25 °C for different HPSEO concentrations. The rate constants were 2.7×10^{-7}, 9.7×10^{-6}, 5.9×10^{-5}, and 6.7×10^{-5} s^{-1} for 2, 3, 4, and 5% HPESO, respectively. The higher of the HPESO concentration was, the faster of the gel formed. The gelation process and stabilizing G' took at least a few hours, which means that the assembly and cross-linking of the HPESO molecules require a fair amount of time to achieve full cure.

After reaching equilibrium at 25 °C, $\geq$2% concentrations of HPESO exhibited viscoelastic solid properties (Fig. 4). For example, the storage moduli (G') curve for the 5% HPESO had a plateau of 1,200 Pa over three frequency decades. The loss moduli (G'') also had a plateau of 160 Pa. The phase shifts were 5.5°–13.8°. The shapes of the G' and G'' curves were very similar to those of gels [10]. 1% HPESO displayed viscoelastic liquid behavior (Fig. 4). Its G'' was greater than G' over the most range of the measured frequency, and the phase shifts were 20°–80°. The moduli curves for 1% HPESO hydrogel were very frequency dependent indicating that the gel was very weak at low concentrations (<2%). However, at 5% HPSEO, the

Fig. 1 The photos of HPESO hydrogel and micrographs. **a** Picture of 5% HPESO, *left* gel formed after reaching equilibrium at room temperature, *right* fluid-like liquid after heating into 55 °C. **b** Microscope photo for 5% HPESO hydrogel at 25 °C, the scale bar is 30 μm

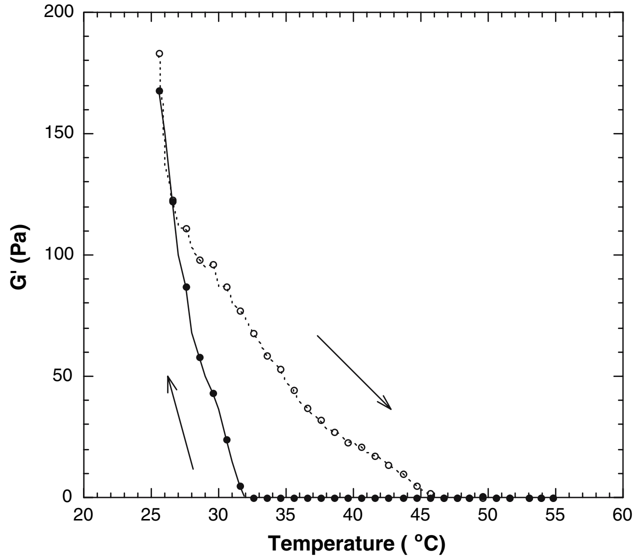


Fig. 2 Thermal reversible behavior of 3% (wt. %) HPESO hydrogel (pH 8.0) with 1 rad/s frequency. *Solid line* and *filled symbols* storage modulus (G') versus temperature during cooling from 55 to 25 °C. *Dashed line* and *open symbols* storage modulus (G') versus temperature during heating from 25 to 55 °C

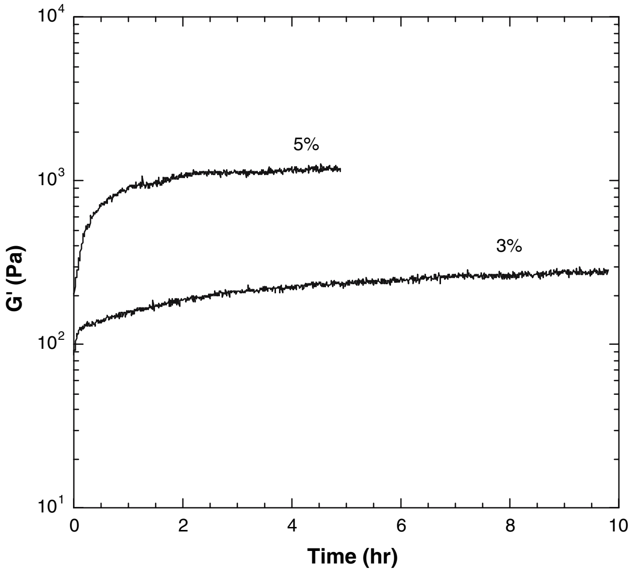


Fig. 3 Storage modulus (G') as a function of time of the gelation procedure for 3 and 5% HPESO hydrogel (pH 8.0) with 1 rad/s and 0.05% strain at 25 °C

moduli curves were nearly independent on frequency, which indicated that the gel should be stronger at higher concentrations (>5%). The shapes of the moduli curves for 3% HPESO were between 5 and 1% HPESO's (Fig. 4). The viscoelastic properties of the HPESO were concentration dependent (Figs. 4, 5). The higher of the concentration, the greater values of G' (Fig. 5). The trend of log(G') (plateau value) versus log(C) followed the Power Law model described for a physical gel [10]. The modulus of HPESO varied as $C^{3.2}$ (Fig. 5), which suggested that HPESO was a physical gel and the concentrations tested were in the range of the near critical concentration since its power index number was higher than two [10].

The experiments described above were repeated at 37 °C and pH 7.4, which are physiological conditions. The same results were achieved, but using higher concentrations of HPESO. For instance, identical results were obtained for 5 and 27% HPESO hydrogels at 25 °C, pH 8.0 and 37 °C, pH 7.4, respectively (data not shown). Thus, much higher concentration of HPESO is needed if a hydrogel for human tissue engineering, which must be made at physiological levels. Therefore, the gel point and gel time for HPESO hydrogels were highly dependent on temperature, pH and concentration. Lower temperature and higher pH reduces the gel point; while greater concentration, lower temperature and higher pH would reduce gel time.

The strain sweep measurements of two concentrations of HPESO are presented in Fig. 6. The linear range of all measured HPSEO concentrations was very small, <0.1% of the shear strain (Fig. 6 shows the results for two HPSEO concentrations), which indicated that the HPESO hydrogel

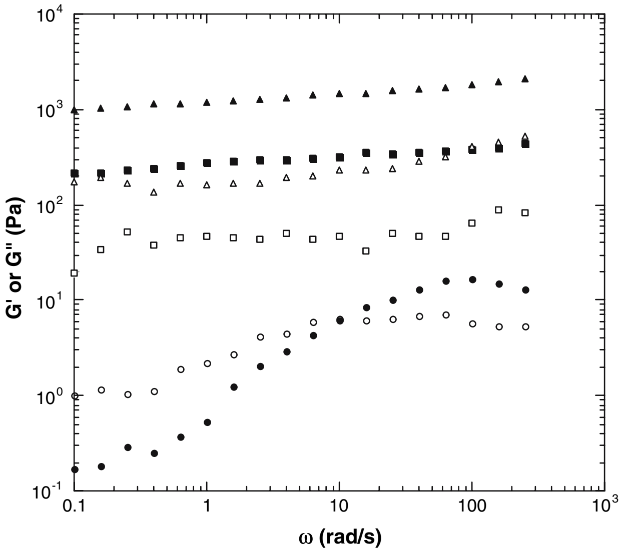


Fig. 4 Linear viscoelastic properties of frequency sweep experiment for HPESO (pH 8.0). Storage modulus (G') or loss modulus (G'') as function of frequency at 25 °C with 0.05% strain. *Filled symbols* G', *open symbols* G''. *Circles* 1% (wt. %) HPESO, *squares* 3% (wt. %) HPESO, *triangles* 5% (wt. %) HPESO

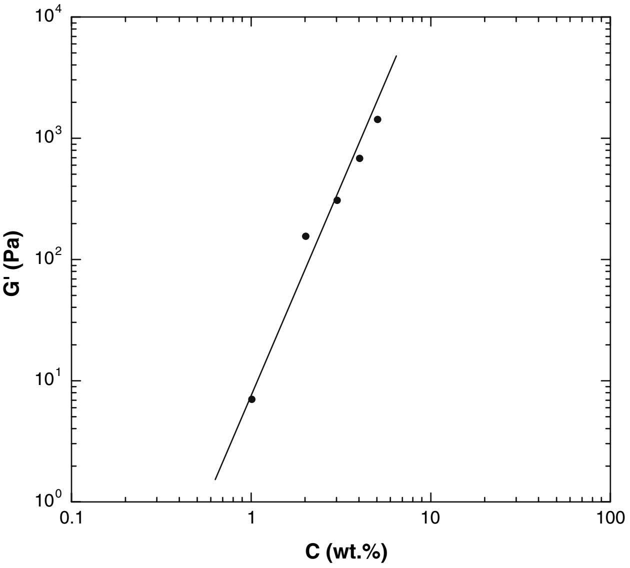


Fig. 5 Plateau values of storage modulus (G') as function of concentration for HPESO (pH 8.0) at 25 °C. The *line* is the linear fitting with a slope of 3.2

was a network “weak” gel [10]. A weak gel is extremely susceptible to disruption, while an entangled “strong” gel can have a linear range to 20% concentration or more. Normally, a hydrogel is formed by a cross-linked network of polymer chains. Cross-linkers can be classified as covalent or physical junctions.

To investigate further whether the HPESO is a physical gel or a chemically cross-linked network, we conducted

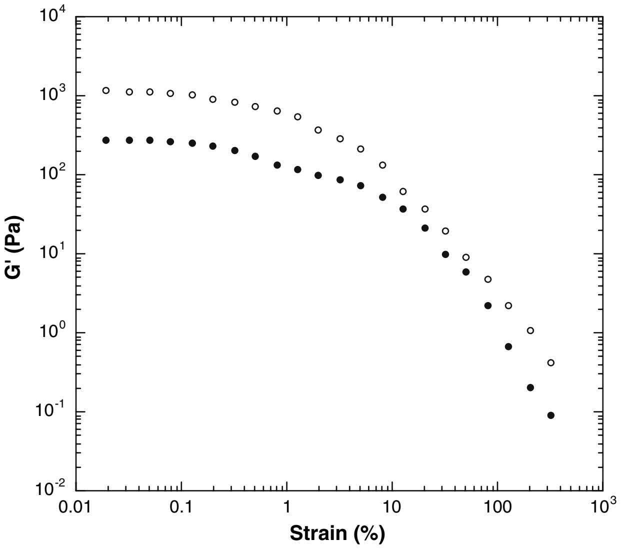


Fig. 6 Strain sweep experiment for 3 and 5% HPESO hydrogels (pH 8.0) with 1 rad/s frequency at 25 °C

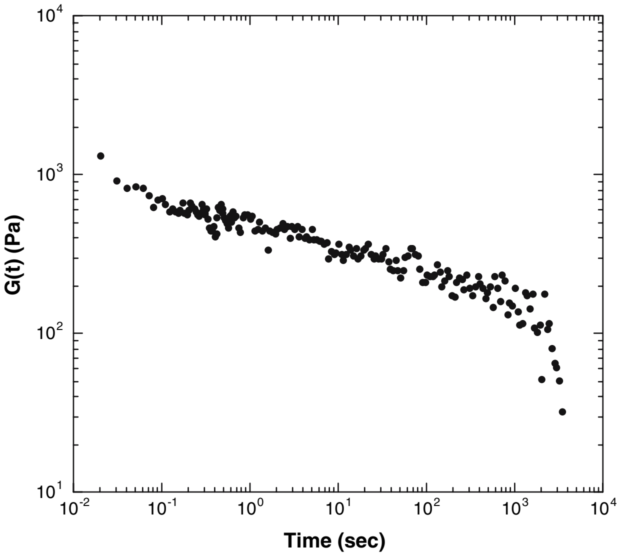


Fig. 7 Stress relaxation measurement for 5% HPESO hydrogel (pH 8.0) after being subject to a 0.05% strain at 25 °C

stress relaxation measurements. The stress relaxation experiment showed that HPESO hydrogel had a long relaxation time. The stress of the 5% HPESO hydrogel relaxed less than two decades within 3000s (Fig. 7). This result indicated that HPESO is a physically cross-linked gel and is not chemically cross-linked even though it has long relaxation time. If a network is tightly cross-linked chemically, there should not be any relaxation and relaxation time should be infinite. This result was also consistent with the conclusion that HPESO hydrogels are physical gels that the Power Law model predicted. In addition, from the stress relaxation measurement, we can estimate of the molecular weight for the cross-linked material from equation $G_e = \rho RT/M_c$ [11], in which G_e, ρ and M_c are the plateau modulus, density, and molecular weight between cross-links, respectively. The plateau modulus for the 5% HPESO hydrogel was about 350 Pa at 25 °C as was obtained from the stress relaxation experiment. Assuming that the density of the material was about 1 g/cm^3, which is the density of water, the molecular weight between cross-links for the 5% HPESO hydrogel was in the order of 10^5 g/mole at 25 °C. This molecular weight was much greater than that of HPESO at 55 °C (ca. 5,000–10,000) [8].

The behavior of thermal reversible assembly disassembly reassembly at physiological pH makes the HPESO hydrogel possess attractive properties for bioengineering application in molecular and cellular encapsulation and in controlled reagent or drug delivery. Another desirable quality for hydrogels is to act as scaffolds for tissue engineering with rapid recoverability in viscoelastic properties after mechanical shearing. In order to test recoverability of HPESO hydrogels, the gelation process was monitored over time after the material being subjected to large shear strain. Figure 8 shows a 5% HPESO hydrogel recovering process after it was under 100% strain deformation at 25 °C. When the material was under a 100% strain, its G' dropped rapidly to 1 Pa from its equilibrium value of 1,200 Pa, and the phase shift jumped from 8° to 77°. Thus, the 5% HPESO hydrogel was almost completely disrupted after 100% strain applied. Within only a few seconds, its G' value recovered to 300 Pa (Fig. 8) and the phase shift was back to 21°. In addition, it took 48 min that the G' recovered into its 50% original equilibrium value; however, the material just recovered about 60% and reached a plateau after 3 h. Although HPESO hydrogels cannot fully recover after mechanical shearing, their rapid initial recovery ability is unique [12]. This capability makes HPESO hydrogels potential candidates for tissue engineering as scaffolds. If the material was heated again to 55 °C and cooled back to 25 °C, after the HPESO hydrogel was disrupted by shearing, it fully recovered indicating the thermally reversible assembly disassembly reassembly of the HPESO hydrogel was not affected. The mechanism of the HPESO molecules assembly disassembly is not clearly understood.

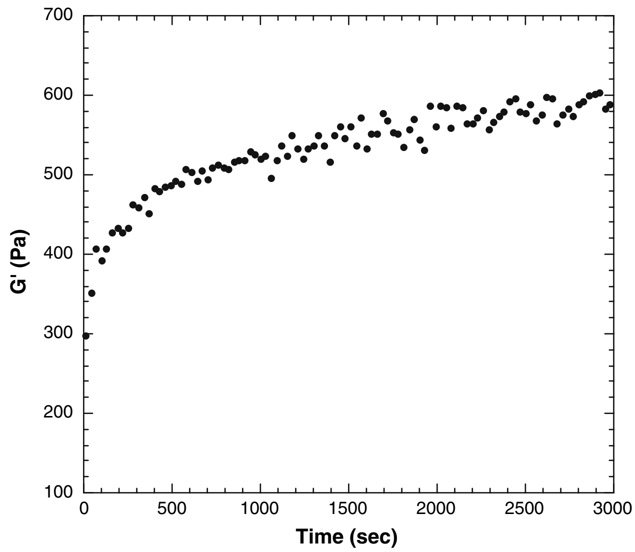


Fig. 8 Recovering storage modulus (G') for 5% HPESO hydrogel (pH 8.0) with 1 rad/s frequency and 0.05% strain after being subject to a 100% shear strain at 25 °C

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References

1. Hoffman AS (2002) Hydrogels for biomedical applications. Adv Drug Deliv Rev 54:3–12
2. Sprung P, Hou Z, Ladin DA (1998) Hydrogels and hydrocolloids: an objective product comparison. Ostomy Wound Manage 44:36–46
3. Cicchetti S, Leone MS, Franchelli S, Santi PL (2002) Evaluation of the tolerability of hydrogel breast implants: a pilot study. Minerva Chir 57:53–57
4. Drury JL, Mooney DJ (2003) Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 24:4337–4351
5. Xu J, Liu ZS, Erhan SZ, Carriere CJ (2002) A potential biodegradable rubber—viscoelastic properties of a soybean oil-based composite. J Am Oil Chem Soc 79:593–596
6. Wool RP, Kusefoglu SH, Khot SN, Zhao R, Palmese G, Boyd A, Fisher C, Bandypadhyay S, Paesano A, Dhurjati P, LaScala J, Williams G, Gibbons K, Wang C, Soultoukis C, Bryner M, Rhinehart J, Robinson A (1998) Affordable composites from renewable sources (ACRES). In: 216th ACS national meeting, Boston, 23–27 August
7. Liu ZS, Erhan SZ, Xu J, Calvert PD (2002) Development of soybean oil-based composites by solid freeform fabrication method: epoxidized soybean oil with bis or polyalkyleneamine curing agents system. J Appl Polym Sci 85:2100–2107
8. Liu Z, Erhan SZ (2006) US Patent application 20070077298
9. Xu J, Bietz JA, Felker FC, Carriere CJ, Wirtz D (2001) Rheological properties of vital wheat gluten suspensions. Cereal Chem 78:181–185
10. Clark AH (1992) "Gels and Gelling" in physical chemistry of foods. In: Schwartzberg HG, Hartel RW (eds) Marcel Dekker, Inc., New York, p 263
11. Bagley EB (1992) "Mechanistic Basis of Rheological Behavior of Foods" in physical chemistry of foods. In: Schwartzberg HG, Hartel RW (eds) Marcel Dekker, Inc., New York, p 573
12. Ramachandran S, Tseng Y, Yu YB (2005) Repeated rapid shear-responsiveness of peptide hydrogels with tunable shear modulus. Biomacromolecules 6:1316–1321