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ORIGINAL ARTICLE

Temperature Dependence of Relaxation Spectra for Self-Assembled Fibrillar Networks of 12-Hydroxystearic Acid in Canola Oil Organogels

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Abstract The temperature dependence, of the mechanical properties of 12-hydroxystearic acid (12HSA) molecular gels, was investigated by means of rheometry and electron microscopy. Application of Tikhonov regularization on the transients of stress relaxation data of 12HSA gels revealed the remarkable impact of fiber morphology on the relaxation spectrum. Relaxation processes with long characteristic times (i.e., τ>10 sec) correspond to thick fibers whereas thin fibers relax on a much faster time scale (i.e., τ<1 sec). Molecular gels crystallized at low temperatures, have variable fiber lengths and widths, show three relaxation events as opposed to the high crystallization temperature counterparts that have uniform fibers and result in fewer relaxation events. These relaxation events correspond exponentially to the fiber cross-sectional thickness.

Keywords Self assembled fibrillar network · Rheology · Organogels · 12 hydroxystearic acid

Introduction

Molecular gels, capable of forming fibrillar networks, have numerous applications including drug delivery, tissue engineering, lipid structuring and scaffolding systems.[1] To illustrate the structural significance of these gels, such as in drug delivery systems, the rate of release is a function of the bioseparation process that corresponds to the mesh size distribution of the network.[2,3] The mesh size is influenced by the rate of nucleation, fiber length as well as the degree of branching and interpenetration.[4] Therefore, the ability to manipulate and control the bioseparation process requires careful considerations of the rheological network properties of the desired gelator–solvent combinations and is crucial in achieving the desired physical properties of these soft materials. Due to the importance of the rheological and network properties, the degree of branching and hence the mesh size distribution have been extensively examined under isothermal[5,6] and non-isothermal conditions.[7–9] The diversity of the microscopic and mesoscopic structures of low molecular weight organogels (LMOG) makes them interesting soft materials with numerous theoretical and practical applications.

The ability of a low molecular weight organogelators (LMOGs) to form rod-like structures is related to a subtle balance among several contrasting parameters, which control the solubility in a given liquid and the epitaxial growth patterns.[10–12] Upon cooling the sol of a self-assembled fibrillar network (SAFiN), it undergoes gelation that is initiated by nucleation of the gelator followed by subsequent crystal growth.[3,6,13] During crystal growth, dissolved gelator molecules, either individually or as aggregates, diffuse and accrete onto a growing crystal surface. Growth may result in one-, two-, or three-dimensional crystals depending on the relative rates at which the dissolved molecules adhere to different surfaces of nucleated species.

Work by Liu has advanced the understanding on how these fibers can be engineered via the modification of the isothermal crystallization conditions of the gelator–solvent

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system.[6] Liu has been instrumental in developing the theory on crystallographic mismatches used to model the branching and generation of permanent junction zones that affect the fiber length and supramolecular structure of these gels. Significant evidence has been provided to support the nucleation–growth–crystallographic mismatch branching (CMB) mechanism (Figure 1). The CMB method suggests that with low degrees of undercooling (ΔT), following nucleation, the fibers grow one-dimensionally with little branching, interpenetration and entanglement.[14] Under non-isothermal crystallization conditions, the driving force of crystallization is a function of both the degree of undercooling (ΔT) and time (Δt), which is a time-temperature parameter (β)[7,8] (Figure 1). At low degrees of bulk supersaturation, the crystallographic mismatch nucleation barrier (ΔG*), which is the energy associated with imperfect incorporation of molecules onto the growing crystal surface, is very high, favoring one-dimensional fiber growth with a corresponding large correlation length (ε). However, when the crystallization temperature is decreased an increase in the supersaturation causes the crystallographic mismatch barrier to be significantly reduced increasing the fiber tip branching.[14] The highly branched fibers (i.e., short correlation length (ε)) coincide with smaller pore sizes.[14] Recently, it has been shown that increased supercooling leads to more elastic gels due to the shorter branched fibers.[7]

Changes in fiber length, thickness, and degree of branching modify the ultrastructure that controls the mechanical response of these and numerous other materials. These changes can be successfully probed using rheometry to investigate the relaxation patterns of molecular events and a useful approach is utilizing the mechanical relaxation spectrum.[15–18] The aim of the present investigation is to elucidate the effect of crystallization parameters on the supramolecular structure of SAFiNs and correlate these modifications of the microstructure to the rheological network properties.

Materials and Methods

Materials

12-hydroxystearic acid (12HSA) was obtained from Sigma-Aldrich (St Louis, MO, USA) and canola oil was obtained from Sunfresh Limited (Toronto, ON, CA) and used as received. Samples were prepared by heating the 12HSA in canola oil (2% (w/w)) to 85 °C for 30 min. The samples were prepared in triplicate and then stored at 30 °C or 5 °C for 24 h prior to microscopy and rheological measurements. All determinations were carried out in triplicate.

Stress Relaxation Measurements and Numerical Computation of the Spectrum

Stress relaxation measurements were performed with an ARG2 Rheometer (TA Instruments, New Castle, DE),

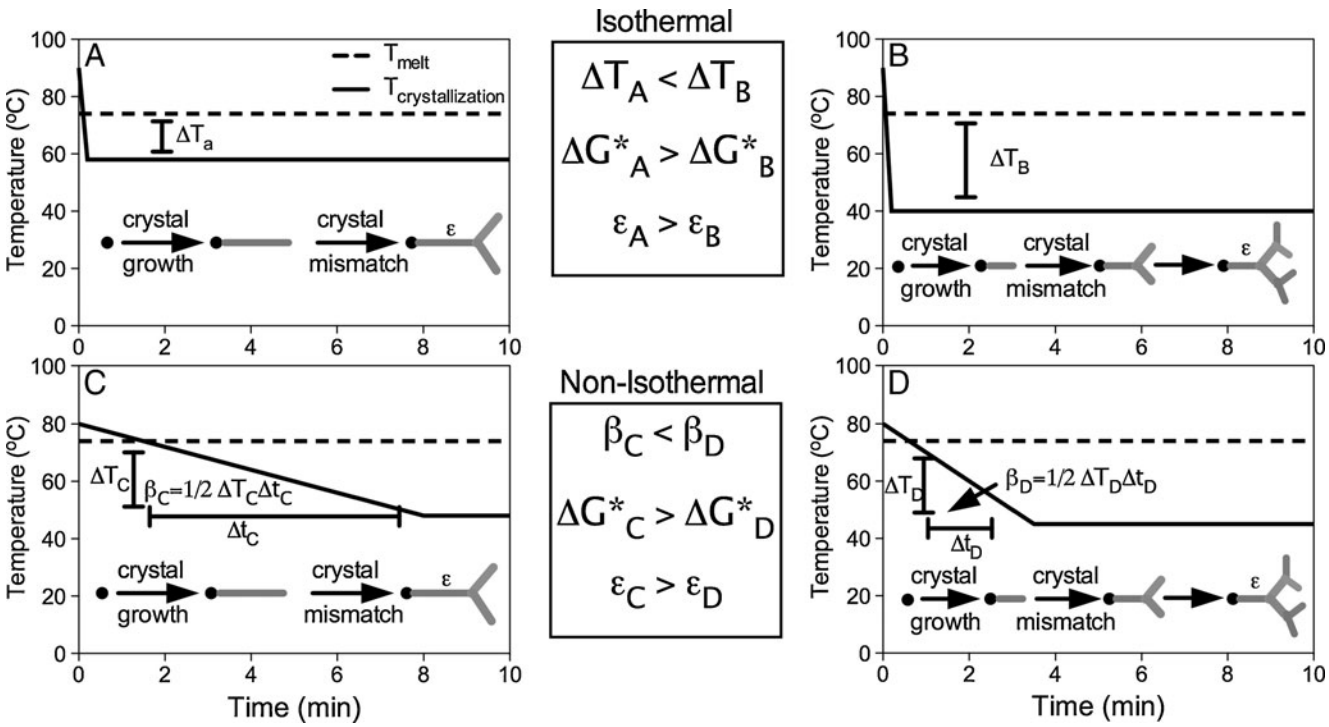

Fig. 1 Diagrammatic representation of isothermal (**a**, **b**) and non-isothermal (**c**, **d**) cooling and the effect of the degree of supercooling (Δt) and the supercooling time parameter (β) on the crystallographic mismatch energy barrier (ΔG*) and the characteristic fiber length (ε)

which is a controlled stress rheometer equipped with a Peltier cooling system for temperature regulation. After an equilibration period of 24 h at 30 °C or 5 °C samples were loaded onto the preheated platen of the rheometer employing serrated parallel plate geometry of 20 mm diameter and 1.5 mm gap.

Stress sweeps in dynamic oscillation on shear were carried out at 5 and 30 °C to identify the linear viscoelastic region (LVR) of the samples at an angular frequency of 1 rad/s. Stress relaxation tests were carried out using the % instantaneous strain falling within the LVR of the material at each different temperature. Fifteen minutes relaxation following application of the instantaneous strain was found to be appropriate to obtain reproducible and highly resolved relaxation spectra. Data of stress relaxation modulus (G(t)) were collected in a logarithmic mode with respect to the timescale of observation.

Numerical computation was performed in MATLAB (v7.0 R14 Service Pack 2, The Mathworks Inc., MA). Discretization of integrals was performed with the *discr.m* script that is published elsewhere.[19] Following creation of matrix *A,* Wendlandt algorithms that employ Tikhonov regularization were used to calculate the optimum regularization parameter (λ=1) and perform data analysis. [20,21]

Cryo-scanning Electron Microscopy of 12HSA/Canola Oil Gels

A drop of molten fat was placed onto a gold coated glass coverslip and stored at 30 °C for 24 h. The sample was placed in a sealed metal container with 0.25 g osmium tetroxide (99.5% pure, Fisher Scientific, Pittsburg, PA) to fix the unsaturated fatty acids in the canola oil. Once the oil was fixed with osmium tetroxide vapors for 1 week at 30 °C the sample was removed and treated with isobutyl alcohol in order to remove unfixed surface oil and to expose the supramolecular network structure. The cover slip was mounted on a copper holder designed for the Emitech K550 Cryo- preparation unit (Ashford, Kent, UK) using Tissue-Tek®. The copper holder was plunged into a liquid nitrogen slush (−207 °C) which was prepared by pulling a vacuum on the liquid nitrogen.

The copper holder was withdrawn from the freezing chamber under an argon blanket to prevent frost from forming on the surface of the samples. They were transferred frozen, and under vacuum into the preparation chamber of the cryo-unit where the sample was sublimated at 80 °C for 30 min. The sample was then coated with 30 nm of gold.

The holders were then transferred from the preparation unit, frozen and under vacuum, onto the SEM (Hitachi S-570, Tokyo, Japan) cold stage held at −137 °C. Images were captured digitally using the Quartz PCI imaging software (Quartz Imaging Corp. Vancouver, BC).

Image analysis was carried out using open source software Image J (Bethesda, Maryland, USA) and the cross-sectional thickness was measured and calibrated to a 100 μm bar. Three images were used and 10 fibers were measured on each electron micrograph. The cross-section of the fibers were subdivided depending on the thickness (i.e., <5 μm; 10 μm to 20 μm; >20 μm).

Results and Discussion

Supramolecular Structure

The resulting supramolecular structure, of self-assembled fibrillar networks (SAFiNs), from solution depends on their thermal history (i.e., how the sample was cooled below the melting temperature).[1–8,13,22] Slowly cooled samples (Figure 1c) or samples that have undergone a small degree of undercooling (Figure 1a) generally develop long fibers (Figure 2b). Samples that have been quickly cooled (Figure 1d) or have undergone a large degree of undercooling (Figure 1b) develop highly branched networks (Figure 2a). Extensive research on the effect of cooling has demonstrated that the microscopic properties of the gel are greatly affected including fiber width, length and pore diameter which results in changes to the macroscopic properties (i.e., turbidity and oil syneresis).[4] These trends correlate with each other because fast-cooled gels or gels which have experienced a large degree of undercooling, undergo numerous crystallographic mismatches reducing the fiber length, crystallinity, pore diameter and finally oil mobility.

However, it is more difficult to evaluate how modifications in the supramolecular structure (i.e., fewer long, thick fibers as opposed to more short, thin fibers) control the rheological behavior. [4] For example, values obtained using compliance measurements indicate higher values for instantaneous compliance (J_o) and retarded compliance (J_1) for gels crystallized at 5 °C compared to 30 °C.[4] However, oscillatory rheological measurements indicate a more elastic gel (i.e., higher elastic modulus (G)) when formed at elevated temperatures.[23] At lower temperatures, the volume of solids (i.e., 12HSA monomers adhered to the crystalline fibers) increases which suggest the elastic modulus should be higher at 5 °C compared to 30 °C.[4] However, the elastic modulus is not only a function of the amount of solids, but also is affected by the spatial distribution of mass, the degree of branching, the crystal perfection as well as numerous other factors. As SAFiNs are cooled rapidly, there is more branching and more crystal imperfections in the network structure which accounts for the lower

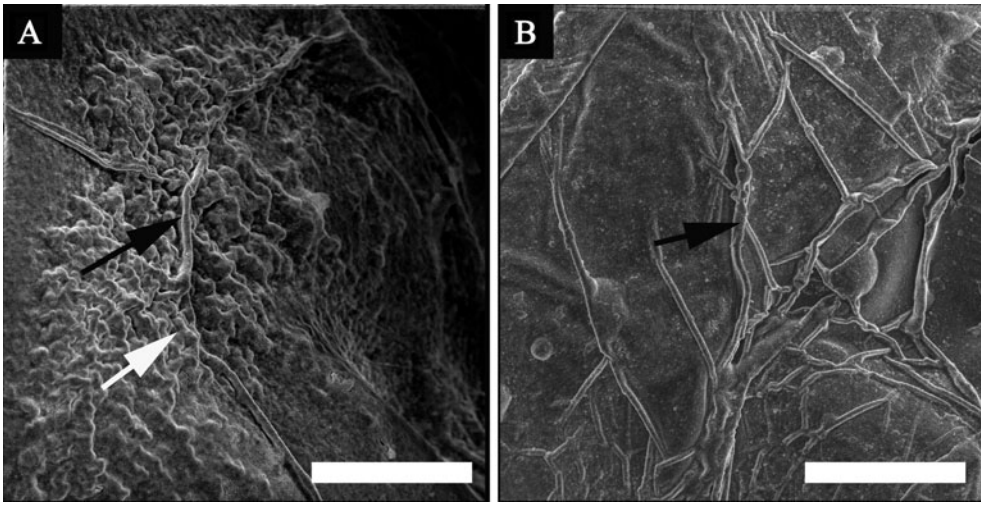

Fig. 2 Scanning electron micrographs of 12HSA in canola oil organogels crystallized at 5 °C (**a**) and 30 °C (**b**). The scale bar is 100 μm. Adapted from ref [2]

elastic modulus.[1] Since these parameters are opposing, it is very difficult to discern the effect of the thermodynamic driving force on the supramolecular network rheological properties. In an attempt to gain better understanding of the intricacies of the aforementioned systems their relaxation behavior was investigated by means of stress relaxation measurements.

Stress Relaxation Measurements

Molecular relaxations of 12HSA networks and their temperature dependence were examined with stress relaxation tests. Since linear viscoelastic range (LVR) varies with temperature it was calculated for all conditions used presently and stress relaxation measurements were performed within the LVR of each sample.

Stress relaxation measurements were obtained and plotted in semi-logarithmic scale (Figure 3). Plotting data in semi-logarithmic mode is necessary so as to determine whether the sample has reached a pseudo-equilibrium G(t) value. Long baselines are necessary for the numerical algorithms to calculate accurately the relaxation spectrum. However, time dependent effects, slippage or aging of the material will change eventually the three-dimensional morphology, hence prolonged experimentation is not feasible. Preliminary measurements showed that data obtained from samples that relaxed for ~15 min are adequate to give highly reproducible spectra. Figure 3 shows that the pseudoequilibrium plateau is reached within 5 min of relaxation, as the modulus remains relatively constant. However, it appears to be little difference between the overall shapes of the curves. Although gels crystallized at 30 °C have a higher elastic modulus than gels crystallized at 5 °C (p=0.05) it is difficult to attribute the increased elasticity to the changes in microstructure. Relaxation spectra that provide additional information on the mechanical behavior of the samples were calculated in the subsequent section using state of the art numerical algorithms.

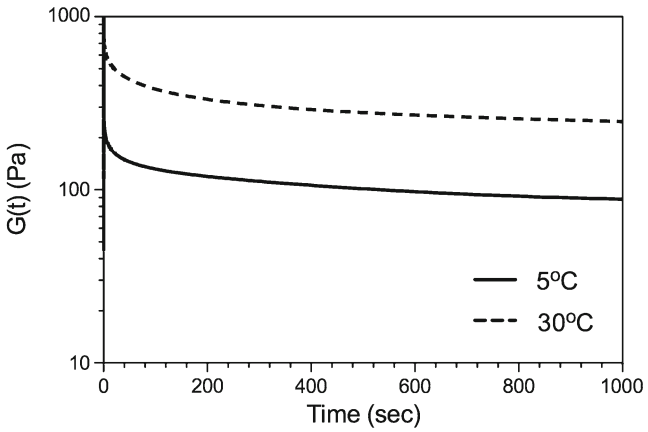

Fig. 3 Stress relaxation curves of 12HSA canola oil organogels for 15 min crystallized at 5 °C and 30 °C

Calculation of Spectra

An experimental methodology to obtain the relaxation spectrum that is frequently used to characterize solid-like viscoelastic specimens is the stress relaxation function on shear in the linear regime. Such a function after sudden application of strain is given by:

$$\sigma(t) = \sigma_e + \int_0^{+\infty} \sigma(\tau) \exp\left(-\frac{t}{\tau}\right) d\tau \quad (1)$$

where σ(t) is the gradual relaxation of stress to the equilibrium stress (σ_e, complete material relaxation means that σ_e=0), with σ(τ) being the distribution function of the elements with relaxation time, τ; the relaxation function σ(τ), therefore, must

be calculated from measurements of σ(t). Numerical methods to solve such problems have been discussed in the literature[24–26] and we have previously described the protocol available for the calculation of relaxation spectra in biopolymer gels[19].

Using Tikhonov regularization to obtain the relaxation spectra reveals drastic differences between the spectra for SAFiNs crystallized at 5 °C and 30 °C (Figure 4). Molecular gels crystallized at 30 °C have two sharp relaxations at 10 and 300 sec while gels produce at 5 °C have three broad relaxations at 0.5, 10 and 300 sec (Figure 4). It is evident that the gels produced at elevated crystallization temperatures produce only thick fibers (indicated by black arrow, Figure 2b). Therefore, when a strain is applied it is difficult for the thick fiber to yield thus long relaxation times are observed. Furthermore, fiber length and fiber thickness are more uniform in molecular gels crystallized at higher temperatures.[2,4] Thus the sharp relaxations observed for gels formed at 30 °C correspond well to previous microscopy data. Conversely, molecular gels crystallized at lower temperatures both have thick fibers (indicated by black arrow) and highly branched, thin fibers (indicated by white arrow, Figure 2a). The formation of the thin, highly branched fibers result in a new relaxation observed at approximately 0.5 sec (Figure 4). As well, the corresponding relaxation spectra at 10 and 300 sec are comparatively broad indicating a wider distribution of fiber lengths and thicknesses. Therefore, relaxation spectra are highly sensitive to fiber morphology in SAFiN comprised on 12HSA in canola oil molecular gels.

Correlation of Microstructure and Relaxation Times

It was found that gels produced at 30 °C had fibers with cross-sectional thickness of 7.5±1.8 μm and 16.2±14.3 μm. There were no fibers, which had a cross-sectional thickness less than 5 μm. Since only two groups of fiber thickness were observed it is likely that the thinner fiber correspond to the shorter relaxation (~8 sec) and the thicker fibers correspond to the longer relaxations (~280 sec). Similarly, the gel produced at 5 °C had similar thickness and relaxations for some fibers (8.3± 2.5 μm with a corresponding 9 sec relaxation and 15.5± 4.3 μm with a corresponding relaxation of 150 sec) however there was a new class of fibers which a short relation around 0.5 sec and a corresponding thickness of 3.1±0.7 μm. There appears to be an exponential correlation between the fiber cross-sectional thickness and stress relaxation time (Figure 5) indicating that the Tikhonov regularization of rheological stress relaxation data provides a powerful tool to correlate the microstructure and rheological structure of complex materials.

Conclusions

Molecular gels comprised of 12HSA in canola oil have similar stress relaxation curves, however, the application of Tikhonov regularization in stress relaxation data indicates that the corresponding relaxation spectra are drastically different. When the molecular gels are crystallized at low temperatures, fiber length and width are highly variable causing the spectra obtained to be broad and more numerous peaks to occur. Conversely, molecular gels formed at high crystallization temperatures have uniform fibers corresponding to less spectra and narrow peaks. Furthermore, thick fibers seem to correspond to long relaxations (i.e., $\tau>10$ sec) while thin fiber relax on a much faster time scale (i.e., $\tau<1$ sec).

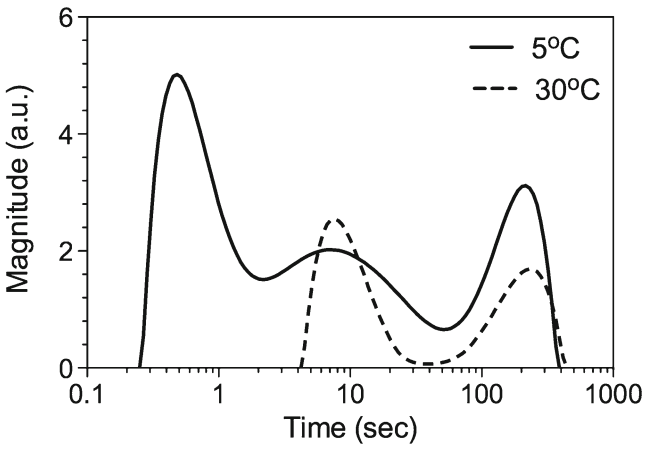

Fig. 4 Temperature dependence of relaxation spectra of 12HSA canola oil organogels at 5 °C and 30 °C exhibiting short and a long-relaxation time regimes

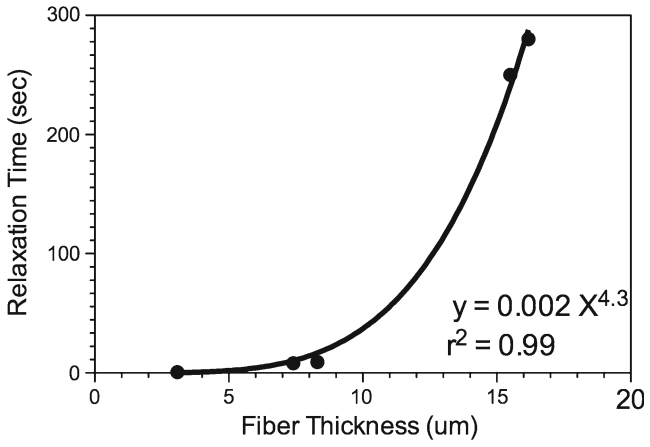

Fig. 5 Relaxation time corresponding to the peak time of the relaxation distribution obtained from the Tikhonov regularization of rheological stress relaxation data verses the corresponding fiber thickness obtained from the electron micrographs

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