ACS Macro Letters

Letter

pubs.acs.org/macroletters

Linear and Nonlinear Rheological Behavior of Fibrillar Methylcellulose Hydrogels

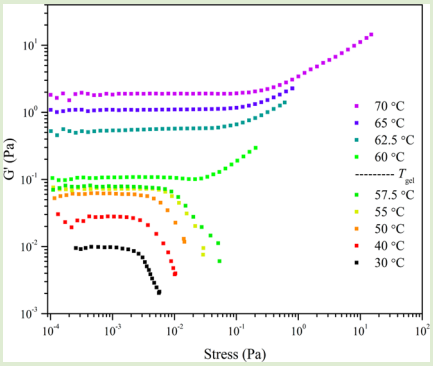
John W. McAllister,[†,⊥] Joseph R. Lott,[†,§,⊥] Peter W. Schmidt,[‡] Robert L. Sammler,[∥] Frank S. Bates,*[,‡] and Timothy P. Lodge*[,†,‡]

[†]Department of Chemistry and [‡]Department of Chemical Engineering & Materials Science, University of Minnesota, Minneapolis, Minnesota 55455, United States

[∥]Materials Science and Engineering, The Dow Chemical Company, Midland, Michigan 48674, United States

Supporting Information

ABSTRACT: Cryogenic transmission electron microscopy and small-angle neutron scattering recently have revealed that the well-known thermoreversible gelation of methylcellulose (MC) in water is due to the formation of fibrils, with a diameter of 15 ± 2 nm. Here we report that both the linear and nonlinear viscoelastic response of MC solutions and gels can be described by a filament-based mechanical model. In particular, large-amplitude oscillatory shear experiments show that aqueous MC materials transition from shear thinning to shear thickening behavior at the gelation temperature. The critical stress at which MC gels depart from the linear viscoelastic regime and begin to stiffen is well predicted from the filament model over a concentration range of 0.18−2.0 wt %. These predictions are based on fibril densities and persistence lengths obtained experimentally from neutron scattering, combined with cross-link spacings inferred from the gel modulus via the same model.



Polysaccharides are among the most versatile polymers produced in nature, providing a wide range of biological functions including structural integrity,[1] energy storage,[2] and immunoresponse.[3] This family of polymers encompasses a diverse set of chemical structures leading to familiar substances such as cellulose, starch, chitosan, and agarose. Each of these natural macromolecules is distinguished by uniquely connected sugar rings and the presence of specific pendant functional groups that together control the wide array of physical characteristics displayed by polysaccharides.[4]

Many natural polymers can be chemically modified, thereby affording the ability to tailor the material properties for a host of commercial uses. Cellulose is a particularly important feedstock where derivatives termed cellulose ethers (CEs) represent a multibillion-dollar market worldwide. One of the simplest CEs, methylcellulose (MC), contains between 1 and 3 methyl ether groups on average per sugar repeat unit, which renders the material partially or fully soluble in water depending on the precise degree of substitution (*DS*) and the detailed distribution of methoxy groups along the chain.[5] MC is used in a wide range of commercial products, often to impart a desirable viscosity in aqueous solutions or an elastic modulus and strength in hydrogels. Particularly relevant is the ability of MC to undergo thermoreversible gelation at elevated temperatures.[6,7] For *DS* values close to two methyl ether groups per anhydroglucose unit, the polymer is water soluble and has a relatively low viscosity at low temperatures (≤20 °C) and end-use concentrations (between the overlap concentration c^* and approximately 2−3% by weight) yet forms a soft gel when heated above 40−70 °C that is accompanied by turbidity and phase separation. Recent experiments demonstrate that gelation and phase separation are contemporaneous, when a single, low heating rate is used.[8,9] In aqueous systems, the gelation temperature can be tuned by adjusting the polymer concentration as well as *DS*.[10]

In recent years there has been a renaissance in the understanding of the structure of MC gels.[11−13] Using a combination of cryogenic-transmission electron microscopy (cryo-TEM) and small-angle neutron scattering (SANS), we have shown that the rheological properties measured in small-amplitude oscillatory shear experiments directly correlate with the growth and dissolution of a fibrillar network upon heating and cooling, respectively. The associated fibrils are remarkably uniform with a diameter of 15 ± 2 nm, independent of MC molar mass, concentration, or temperature of gel formation. This discovery represents a qualitative departure from previous interpretations of rheological experiments, where an entanglement network has often been invoked.[14−16] In this report, we demonstrate that the linear and nonlinear rheological properties of MC gels can be understood in terms of an established model for elastic gels comprising a network of filaments.

We apply the mechanical model developed by MacKintosh et al.[17] to aqueous MC gels. Originally developed to describe the elasticity of actin solutions and gels, it has more recently been

Received: February 26, 2015
Accepted: April 16, 2015
Published: April 21, 2015

DOI: 10.1021/acsmacrolett.5b00150
ACS Macro Lett. 2015, 4, 538−542

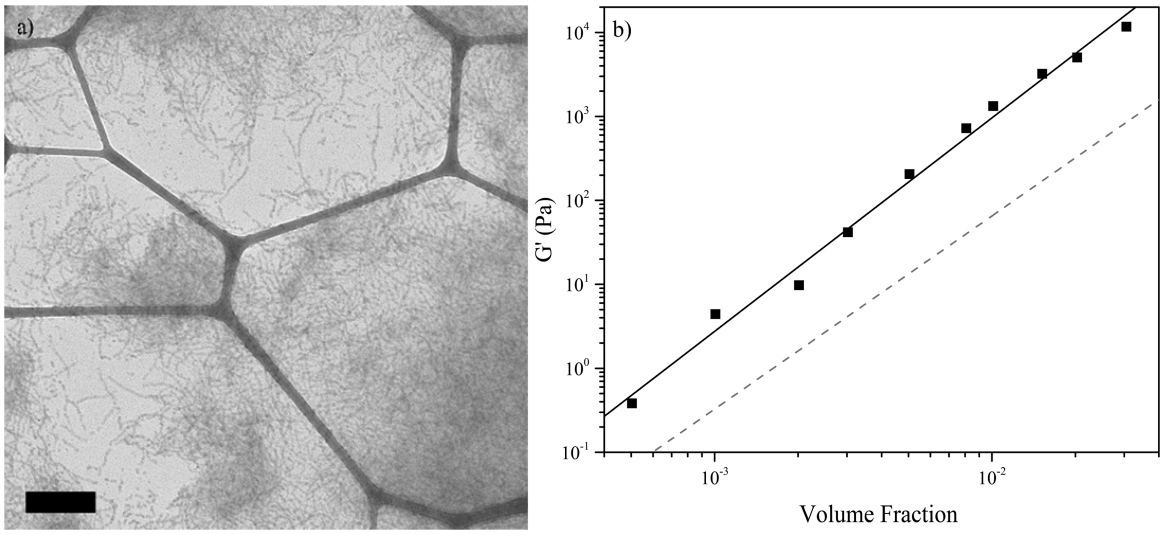


Figure 1. (a) Cryo-TEM image of an aqueous 0.3 wt % MC-300 sample vitrified from 70 °C (scale bar = 500 nm) supported on a lacey carbon support grid. (b) Storage modulus versus polymer concentration at 70 °C (1 rad/s).[9] The solid line is the power law best fit to the data. The gray dashed line represents an extrapolation of the plateau modulus of polyethylene, one of the most highly entangled flexible polymers.

applied to interpret a variety of other biopolymer systems such as neurofilaments,[18] gels from self-assembled β-hairpins[19] or bile acid analogues,[20] and fibrin protofibrils.[21] The elasticity of the network derives from the force response of a single filament, which in turn is based on the worm-like chain model.[22] The reduction in conformational entropy upon stretching a semiflexible filament generates a restoring force in opposition to the network tension created by deformation. A macroscopic network of such filaments is modeled as an isotropic array of random nodes that connect the filaments. These nodes, which play the role of cross-links, are not permitted to support torques.[21] While more sophisticated models dealing with the nonlinear elasticity of flexible and semiflexible networks have been reported recently,[23,24] we have chosen to employ the simpler approach since it utilizes parameters such as fibril persistence length, filament density, and radius that have been independently measured for MC hydrogels using SANS and cryo-TEM.

A cryo-TEM image obtained after vitrifying a 0.3 wt % MC (M_W = 300 000 g/mol, MC-300, DS = 1.80)[25] gel formed at 70 °C is shown in Figure 1a, clearly evidencing a fibrillar morphology. Isothermal rheological experiments, interpreted using the Winter−Chambon gelation criterion, along with isochronal measurements of G' and G'' while heating (Figure S1, Supporting Information) establish that the material is fully gelled at 70 °C. The fibrils evident in this cryo-TEM image have an average diameter of 16 ± 2 nm, in agreement with prior reports.[11−13] Regions within the image that appear darker either reflect variations in sample thickness or local clustering of MC, similar to what has been reported earlier.[12] Also shown in Figure 1b is the dynamic elastic modulus G' obtained at 70 °C with a frequency of 1 rad/s in the linear regime, plotted as a function of the volume fraction of MC, determined assuming a density of 1.39 g/mL for the polymer.

The model developed by MacKintosh et al. makes several predictions that are consistent with the behavior of MC-based gels. Solutions of filaments are predicted to display a storage modulus that scales with concentration as $c^{11/5}$, arising from the geometric constraints imposed by entanglements; the modulus of highly cross-linked filamentous gels increases as $c^{5/2}$.[17] Figure 1b demonstrates that $G' \sim c^{2.5}$, consistent with our previously reported scaling, $G' \sim c^{2.3}$ (at 80 °C); others have reported scaling exponents ranging from 1.3 to 3.0.[9,26] Differences in experimental approaches (e.g., frequency vs temperature sweeps) lead to varying thermal histories that can significantly affect the mechanical properties of the gels and the resulting concentration dependence. In addition, T_{gel} decreases with increasing polymer concentration. Therefore, selecting a single reference temperature (70 °C in this case) leads to variations in the thermal offset with respect to T_{gel}, for samples with different concentrations. However, in the present work we justify this tactic as extensive characterizations of the fibrillar gels have been carried out at this temperature for a large range of concentrations of MC gels. The main point, however, is that the concentration dependence alone cannot confirm or refute the inference that the gel moduli are derived from the fibrillar structure, as many systems display similar dependences. To illustrate this point, Figure 1b also contains an extrapolation of the entanglement modulus of polyethylene (PE) in solutions and melts.[27] It is clear that a similar scaling with concentration is observed. However, the greater magnitude of the MC modulus compared to PE, by roughly an order of magnitude, demonstrates that MC gelation cannot be a consequence of immobilized entanglements, unless one adopts the unphysical view that MC has a molecular weight between entanglements in the bulk corresponding to about one anhydroglucose residue.

Linear viscoelastic measurements have dominated rheological investigations of MC gels, but it is in the nonlinear regime that filamentous gels display more distinguishing traits.[28,29] We have used large-amplitude oscillatory shear experiments to probe the transition from shear thinning to shear thickening behavior as the gelation temperature is traversed. Figure 2 contains results for these experiments for both the lowest and highest concentrations investigated in this study (further data are provided in Figure S2, Supporting Information). Below the gelation temperature (T_{gel}), the samples exhibit shear thinning at high stresses, typical of polymer solutions and usually ascribed to partial alignment of chains with the flow field. However, once T_{gel} is reached or exceeded, the plateau modulus rapidly increases as temperature is further raised, consistent

DOI: 10.1021/acsmacrolett.5b00150
ACS Macro Lett. 2015, 4, 538−542

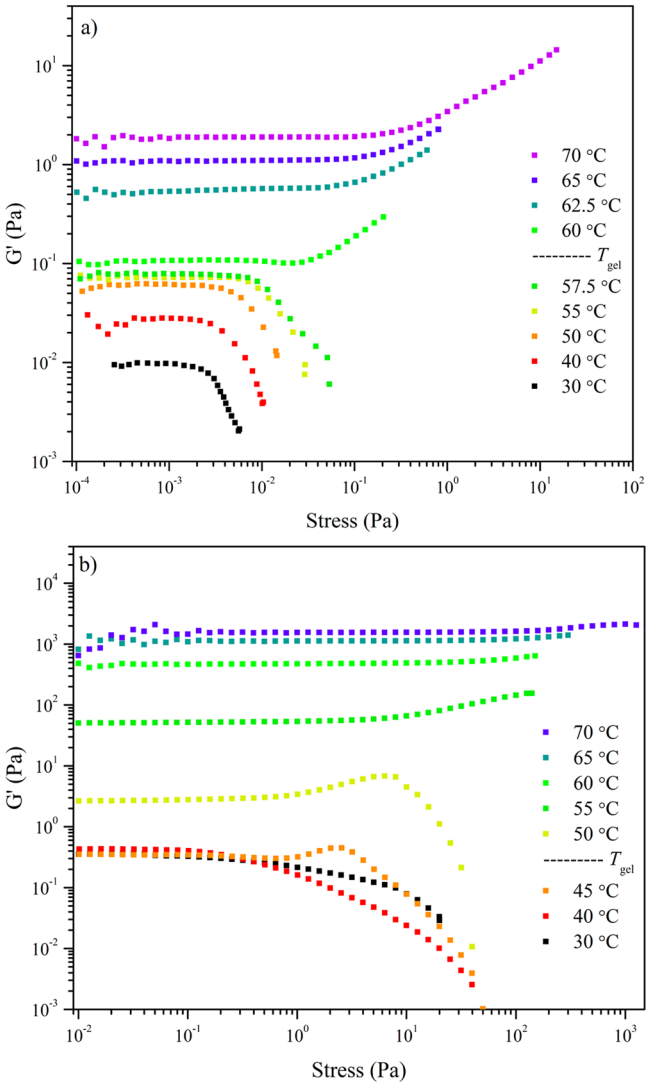


Figure 2. Dynamic storage modulus versus stress measured at 1 rad/s at discrete temperatures: (a) 0.18 wt % MC-300 and (b) 2.0 wt % MC-300.

with gelation. At high oscillatory stresses the samples display shear thickening, with the stress corresponding to the onset of nonlinearity increasing with temperature. This flow-stiffening behavior is a hallmark of filamentous biopolymer gels and is inconsistent with the typical behavior of gels comprised of flexible polymers. Mechanistically, the behavior arises due to the fact that filaments quickly become stretched to a nearly straight conformation at relatively low strains since the semiflexible nature inherently limits conformational features such as loops. Additionally, this behavior, attributable to the individual filaments, means that a rapid, nonlinear force response to extension far outpaces the resistive forces to compression, for equal strains. For filaments that are isotropically oriented, shearing will activate roughly equal numbers of filaments in compression versus tension. The imbalance of restoring forces means that that filamentous/fibrillar gels will display a negative first normal stress difference, as is observed (Figure S3, Supporting Information).[30] This is also in direct contrast to the behavior of gels composed of flexible polymer chains and further supports the notion that the bulk mechanical properties reflect the fibril structure.

The relationship between the modulus (G_0) and the structural features of the fibrillar gel, such as the fibril density (ρ, length per volume), persistence length (l_p), and the distance between cross-links (l_c) is given by[18]

$$G_0 = 6\rho k_B T \frac{l_p^2}{l_c^3} \quad (1)$$

where k_B is Boltzmann's constant and T is temperature. We have previously elucidated the dimensions and composition of fibrillar MC gels using small-angle neutron scattering (SANS). As noted above, fibrils form with a diameter of 15 ± 2 nm regardless of MC concentration and M_w. These fibrils have a persistence length of approximately 30 nm and contain 40% polymer by volume (Figures S4−6, Supporting Information), assuming that essentially all the MC is in fibrils.[12,13] These structural features are essential ingredients in applying the model discussed herein. For example, the effective fibril density (Figure 3a) is increased due to the presence of solvent within the fibril according to eq 2

$$\rho = \phi_p / x\pi r^2 \quad (2)$$

where ϕ_p is the polymer volume fraction: x is the volume fraction of polymer within the fibril; and $2r$ is the fibril diameter. Combined with the modulus values measured in the current work, eq 1 can be used to calculate the average distance between cross-linking nodes in the gels, which are displayed in Figure 3b. For gel concentrations of practical interest, the values for l_c are on the order of 10−100 nm, broadly consistent with what is observed in Figure 1a.

Quantification of the distance between fibril cross-links based on cryo-TEM images should be avoided since these represent 2D projections of a 3D gel network. In fact, precise values for l_c cannot be readily determined experimentally, so the values in Figure 3b provide a level of insight previously lacking in MC gels. However, we have previously noted that network dimensions of the scale shown in Figure 3b do not satisfactorily account for the optical turbidity observed in the gel state and that larger-scale (≥500 nm) heterogeneities also exist in the gel network.[8,13] This also has implications when considering the values extracted from the mechanical model that assumes the fibrils form a defect-free network. For a given value of the modulus, the presence of large low concentration "defects" in the gel[31,32] means that the local mesh will likely have smaller values of l_c than calculated here. The comparable size of the measured persistence lengths and calculated distances between cross-links ($l_p \approx l_c$) is also confirmed by a successful fit to the model proposed by Dobrynin et al.[23,24] (see Figure S7, Supporting Information), which gives the ratio of the mean-square end-to-end distance of the unperturbed filament to the mean-square end-to-end distance of a fully stretched filament, $\langle R_{in}^2 \rangle / \langle R_{max}^2 \rangle = 0.93$. This provides further support to the validity of approaches to MC materials based on semiflexible models.

Most importantly, the current treatment allows for the prediction of the critical stress (σ_c) at which the rheological behavior deviates from linearity[18]

$$\sigma_c = \rho k_B T \frac{l_p}{l_c^2} \quad (3)$$

Figure 4 contains LAOS stress amplitude sweeps for MC gels at 70 °C with a concentration range spanning from 0.18 to 2.0 wt

DOI: 10.1021/acsmacrolett.5b00150
ACS Macro Lett. 2015, 4, 538−542

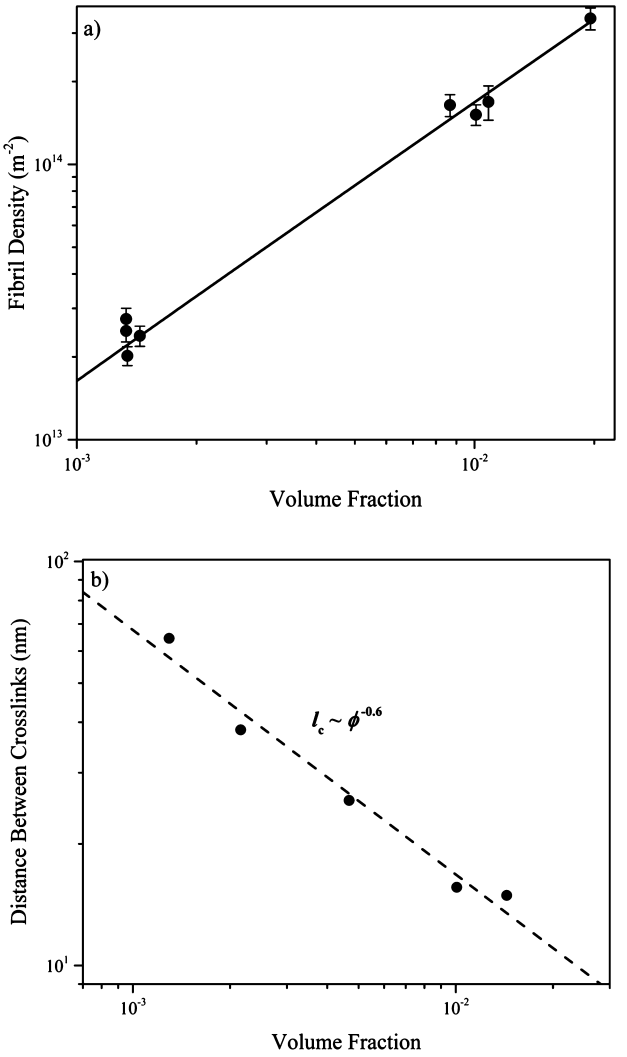


Figure 3. (a) Calculated fibril density as a function of volume fraction using eq 2. The data points are based on SANS data previously reported.[13] The black line shows the linear fit to the calculated points. (b) Calculated distance between cross-links as a function of volume fraction for MC gels at 70 °C, using eq 1. For the concentrations of the samples used in the current rheological investigation, no data for persistence length and fibril density are available. In order to calculate the distances, general relationships for these variables as a function of concentration (Figure 3a and Figure S5, Supporting Information) were utilized in combination with the measured plateau moduli for the concentrations displayed as points. The dashed line is a linear fit to these calculated points.

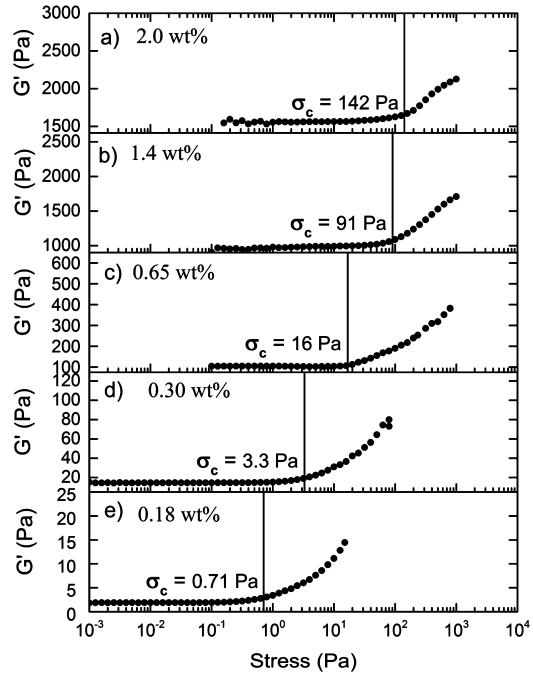


Figure 4. Storage modulus versus oscillatory stress for aqueous MC-300 gels at 70 °C of varying concentrations. The samples were measured in stress-controlled mode with a frequency of 1 rad/s. The vertical lines are the predictions of the critical stress for each sample from eq 3.

%, along with the predicted values for σ_c from eq 3. As the concentration of the gels increases, nonlinearity sets in at higher stresses (yet lower strains).

It is apparent that eq 3 affords very satisfying predictions for stresses that range over almost 2 orders of magnitude. This is remarkable in light of several considerations. As previously discussed, at 70 °C, each concentration is a different distance in temperature from the associated T_{gel}. In addition, the exact nature of the cross-links within MC is not yet known and a matter of conjecture given that hydrophobic interactions, interfibril hydrogen bonding, and simple entanglements may all play a role.[33] The model is based on pin joints (which do not allow for bending forces to be exerted through the node), yet rotating joints or welded joints may be more appropriate once the true nature of the cross-links are elucidated.[34] This model assumes that fibrils are uniformly distributed throughout the network, yet the optical turbidity of the gels indicates this is not the case. Furthermore, the heterogeneity of the internal structure of the fibrils means there may be some internal compliance to tension within the fibrils themselves, which is absent in the mathematical description of the individual filaments.

We have found that a mechanical model for probing the mechanical properties of filamentous gels can be applied to the new paradigm of fibrillar MC gels. As opposed to other biological systems that are highly sensitive to pH and other factors, MC may offer a facile and robust platform to experimentally probe the application of filament-based mechanical models. Interestingly, despite the enormous extent of heterogeneity in the system (amount and regiospecificity of methoxy substitutions, broad M_w distributions, dispersity of fibrillar radii and contour lengths, multiple possible mechanisms for cross-linking (hydrogen bonding, hydrophobic interactions, fibril entanglements), and heterogeneity in cross-link functionality and density) MC gels can still serve as powerful model polymer systems. Unresolved questions regarding the mechanism of MC gelation and the detailed fibrillar structure of MC gels will ensure continued interest in these fascinating materials.

EXPERIMENTAL SECTION

The methylcellulose used in this study was provided by The Dow Chemical Company under the trade name of METHOCEL.[35] As previously reported, this sample has M_w = 300 kDa, $Đ$ = 5.4, and DS = 1.8.[9] Aqueous solutions were prepared by suspending the dried powder in distilled water at 80 °C and magnetically stirring for 15 min. This was followed by addition of room-temperature water to arrive at the desired final concentration and stirring for 15 min, at which time the flask was lowered into an ice bath for an additional 15 min of

DOI: 10.1021/acsmacrolett.5b00150
ACS Macro Lett. 2015, 4, 538−542

stirring. The resultant polymer solutions were stored in a refrigerator (4 °C) for a minimum of 12 h prior to use. Cryogenic transmission electron microscopy (cryo-TEM) experiments were conducted within the University of Minnesota Characterization Facility using methods and procedures previously reported.[12] Rheological testing was performed on a stress-controlled TA Instruments AR-G2 rheometer using a parallel plate geometry (diameter = 40 mm, gap = 350−550 μm). Evaporation of the solvent was minimized by placing a low viscosity silicon oil around the sample perimeter. Stress amplitude sweeps were conducted at discrete temperatures, and care was taken to select a stress range at each temperature that captured the nonlinear response but did not fracture the gels. Samples were held at the desired test temperature for 15 min prior to measuring the strain response, and each isothermal stress amplitude sweep required about 10 min to complete, which is much less time than the slow nucleation and growth of MC gels in the temperature range between 45 and 60 °C. Additionally, rheological measurements were rapid compared to the very slow aging of the gel structure at 70 °C.[36]

■ ASSOCIATED CONTENT

Supporting Information

Additional rheology data and SANS data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: bates001@umn.edu.

*E-mail: lodge@umn.edu.

Present Address

[§](J.R.L.) School of Polymers & High Performance Materials, University of Southern Mississippi, Hattiesburg, MS 39406.

Author Contributions

[⊥](J.W.M. & J.R.L.) These authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Dr. Fred C. MacKintosh for insightful conversations concerning the rheological behavior of filamentous gels. We would also like to thank Dr. Sara Arvidson for graciously providing rheological data. This work was funded by a business unit (Dow Pharma and Food Solutions) of The Dow Chemical Company. Cryo-TEM measurements were carried out in the CSE Characterization Facility, University of Minnesota, which receives partial support from NSF through the MRSEC program.

■ REFERENCES

(1) Shukla, S. K.; Mishra, A. K.; Arotiba, O. A.; Mamba, B. B. *Int. J. Biol. Macromol.* **2013**, *59*, 46.

(2) Prajapati, V. D.; Jani, G. K.; Moradiya, N. G.; Randeria, N. P.; Nagar, B. J.; Naikwadi, N. N.; Variya, B. C. *Int. J. Biol. Macromol.* **2013**, *60*, 83.

(3) Cummings, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 9669.

(4) Wen, Y.; Oh, J. K. *Macromol. Rapid Commun.* **2014**, *35*, 1819.

(5) Denham, W. S.; Woodhouse, H. J. *Chem. Soc. Trans.* **1913**, *103*, 1735.

(6) Heyman, E. *Trans. Faraday Soc.* **1935**, *31*, 846.

(7) McKee, J. R.; Hietala, S.; Seitsonen, J.; Laine, J.; Kontturi, E.; Ikkala, O. *ACS Macro Lett.* **2014**, *3*, 266.

(8) Fairclough, J. P. A.; Yu, H.; Kelly, O.; Ryan, A. J.; Sammler, R. L.; Radler, M. *Langmuir* **2012**, *28*, 10551.

(9) Arvidson, S. A.; Lott, J. R.; McAllister, J. W.; Zhang, J.; Bates, F. S.; Lodge, T. P.; Sammler, R. L.; Li, Y.; Brackhagen, M. *Macromolecules* **2013**, *46*, 300.

(10) Sarkar, N. *Carbohydr. Polym.* **1995**, *26*, 195.

(11) Bodvik, R.; Dedinaite, A.; Karlson, L.; Bergstrom, M.; Baverback, P.; Pedersen, J. S.; Edwards, K.; Karlsson, G.; Varga, I.; Claesson, P. M. *Colloids Surf., A* **2010**, *354*, 162.

(12) Lott, J. R.; McAllister, J. W.; Arvidson, S. A.; Bates, F. S.; Lodge, T. P. *Biomacromolecules* **2013**, *14*, 2484.

(13) Lott, J. R.; McAllister, J. W.; Wasbrough, M.; Sammler, R. L.; Bates, F. S.; Lodge, T. P. *Macromolecules* **2013**, *46*, 9760.

(14) Kobayashi, K.; Huang, C.; Lodge, T. P. *Macromolecules* **1999**, *32*, 7070.

(15) Li, L.; Thangamathesvaran, P. M.; Yue, C. Y.; Tam, K. C.; Hu, X.; Lam, Y. C. *Langmuir.* **2001**, *17*, 8062.

(16) Chatterjee, T.; Nakatani, A. I.; Adden, R.; Brackhagen, M.; Redwine, D.; Shen, H.; Li, Y.; Wilson, T.; Sammler, R. L. *Biomacromolecules* **2012**, *13*, 3355.

(17) MacKintosh, F. C.; Käs, J.; Janmey, P. A. *Phys. Rev. Lett.* **1995**, *75*, 4425.

(18) Yao, N. M.; Broedersz, C. P.; Lin, Y.-C.; Kasza, K. E.; MacKintosh, F. C.; Weitz, D. A. *Biophys. J.* **2010**, *98*, 2147.

(19) Ozbas, B.; Rajagopal, K.; Schneider, J. P.; Pochan, D. J. *Phys. Rev. Lett.* **2004**, *93*, 268106−1.

(20) Terech, P.; Sangeetha, N. M.; Maitra, U. *J. Phys. Chem. B* **2006**, *110*, 15224.

(21) Storm, C.; Pastore, J. J.; MacKintosh, F. C.; Lubensky, T. C.; Janmey, P. A. *Nature* **2005**, *435*, 191.

(22) Kratky, O.; Porod, G. *Recl. Trav. Chim. Pays-Bas.* **1949**, *68*, 1105.

(23) Dobrynin, A. V.; Carrillo, J.-M. Y. *Macromolecules* **2011**, *44*, 140.

(24) Carrillo, J.-M. Y.; MacKintosh, F. C.; Dobrynin, A. V. *Macromolecules* **2013**, *46*, 3679.

(25) Methylcellulose. *United States Pharmacopeia and National Formulary*; United Book Press, Inc.: Baltimore, MD, 2012; Vol. *35*, pp 3868−3869.

(26) Li, L. *Macromolecules* **2002**, *35*, 5990.

(27) Tao, H.; Lodge, T. P.; von Meerwall, E. D. *Macromolecules* **2000**, *33*, 1747.

(28) Wen, Q.; Basu, A.; Winer, J. P.; Yodh, A.; Janmey, P. A. *New J. Phys.* **2007**, *9*, 428.

(29) Kang, H.; Wen, Q.; Janmey, P. A.; Tang, J. X.; Conti, E.; MacKintosh, F. C. *J. Phys. Chem. B* **2009**, *113*, 3799.

(30) Janmey, P. A.; McCormick, M. E.; Rammensee, S.; Leight, J. L.; Georges, P. C.; MacKintosh, F. C. *Nat. Mater.* **2007**, *6*, 48.

(31) Jee, A.; Curtis-Fisk, J. L.; Granick, S. *Macromolecules* **2014**, *47*, 5793.

(32) Ruta, B.; Czakkel, O.; Chushkin, Y.; Pignon, F.; Nervo, R.; Zontone, F.; Rinaudo, M. *Soft Matter.* **2014**, *10*, 4547.

(33) Huang, W.; Dalal, I. S.; Larson, R. G. *J. Phys. Chem. B* **2014**, *118*, 13992.

(34) Picu, R. C. *Soft Matter.* **2011**, *7*, 6768.

(35) Trademark of The Dow Chemical Company.

(36) Schupper, N.; Rabin, Y.; Rosenblug, M. *Macromolecules* **2008**, *41*, 3983.

DOI: 10.1021/acsmacrolett.5b00150
ACS Macro Lett. 2015, 4, 538−542