Auburn University Journal of Undergraduate Scholarship

Addition of Self-assembling Small Molecules to Cellulose Hydrogels to Enhance Gelation and Tune Microstructure

Jordan N. Clemmons[1,] , Symone Alexander[2]*

[1,*] Undergraduate Student, Department of Chemical Engineering, Auburn University
[2]Assistant Professor, Department of Chemical Engineering, Auburn University

Chronic kidney disease (CKD) affects 1 in 7 U.S. adults (CDC). 71% of those with CKD are treated with dialysis (NIDDK), which lowers the quality of life for patients due to the long treatment times and the stress it puts on the patient's body. In this work, orally administered sorbent microgels synthesized from cellulose are being explored as an alternative treatment to NIDDK. These dual-functioning microgels mimic dietary fiber by absorbing uremic toxins while releasing probiotics.

Cellulose is an abundant organic compound found in plants [1]. Due to cellulose being natural and biodegradable, it is the perfect matrix material for gels and microgels. Cellulose microgels have many advantages including tunable pore size for diffusion, large surface area, and an adaptive shape. These characteristics make cellulose microgels useful for absorbing materials and carrying active substances through the body [7].

However, a few challenges with these gels include a lengthy gelation time and variable microstructures, which affects the diffusion of materials into and out of the microgels [6,8]. Self-assembling small molecules can be added to cellulose gels to tune the microstructure of the gel and ensure the correct pore size and geometry [2,3]. The addition of these small molecules can also decrease the gelation time due to rapid self-assembly. In this study, we investigate the gelation characteristics of an Fmoc-derivative that is a pH-responsive small molecule and its effect on cellulose-based microgel structure.

Cellulose is soluble at low temperatures in high pH sodium hydroxide (NaOH) solutions, while the small molecule used in this study self-assembles to form a gel at high pH; by mixing these solutions, we form a sacrificial template (small-molecule gel) for cellulose gel formation at high pH. The small molecule used is mono-Fmoc ethylene diamine hydrochloride, which contains the Fmoc protecting group. The Fmoc protecting group is widely used to synthesize small molecule gels and helps with self-assembly due to π-π stacking and structural packing arrangements [4]. Additionally, the Fmoc group is connected to a hydrophobic peptide chain that leads to gelation at high pH.

To fabricate the most effective gel, the stability of Fmoc at different pH levels was investigated. The pH level also corresponds to NaOH concentration and, for a strong base, significant changes in concentration are needed to decrease the pH. Fmoc gels synthesized in pH ranging from 12.5 to 12.9 were fabricated by dissolving 0.5 mg of mono-Fmoc ethylene hydrochloride in 1 mL of DI water. Different concentrations of NaOH solutions were calculated using equations (1) - (3), then mixed with the Fmoc solution to create the gels at different pH levels. The vial inversion test was then used to determine whether a gel was formed [2,5].

$$M_1V_1 = M_2V_2 \tag{1}$$

$$pOH = -\log[OH^-] \tag{2}$$

$$pH = 14 - pOH \tag{3}$$

The gel at the lowest pH, 12.5, did not fully gel and flowed during the vial inversion test. The gels at a higher pH were noticeably more opaque and maintained stability and position when inverted (Figure 1). This leads to the conclusion that Fmoc forms a more uniform gel as you increase the pH.

---

* Corresponding author: jnc0050@auburn.edu

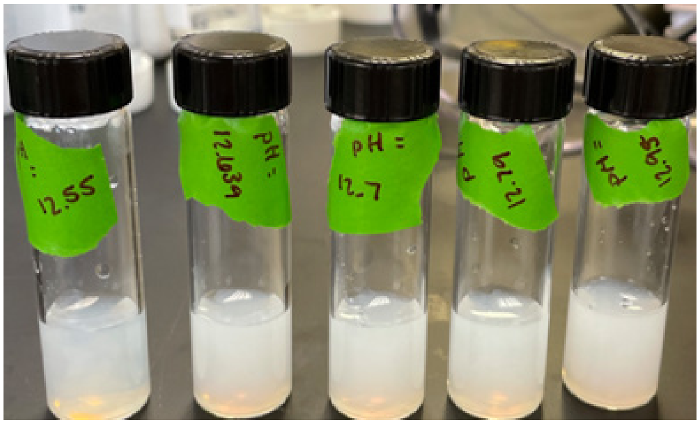

Fig 1. NaOH and Fmoc gels immediately after gelation.

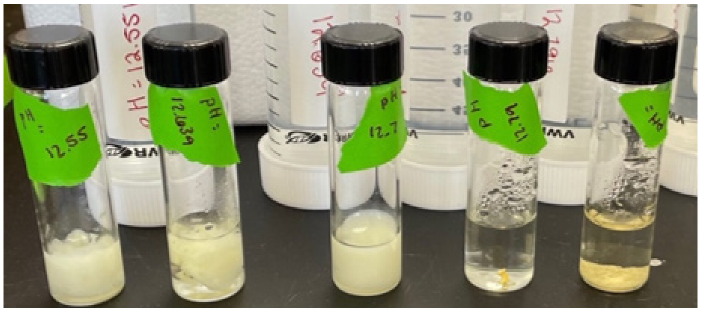

Fig 2. NaOH and Fmoc gels after a month

Following the effects of the pH on gelation, the stability of the gel over time was investigated (Figure 2). The gel at the highest pH broke down the fastest with the gel showing decomposition after 3 weeks. The gel at the lower pH also broke down quickly with decomposition present at 3 weeks, but with more gel present than in the higher pH. In contrast, the gels at a pH of 12.7 remained the most stable with the gel still uniform after 1 month, and the gel even remained after 2 months. From this, it can be determined that the Fmoc also destabilizes at a certain high pH, leading to the breakdown of the gel [10]. On the other hand, the lower pH gel behavior can be explained by the Fmoc not fully forming a gel when the pH is lowered to a certain pH.

Light microscopy was used to determine the effect of pH on the gel structure. The microscopy images showed an increase in the density and connectivity of fiber networks that was proportional to an increase in pH, as shown in Figure 3. In Figure 3, (A) showed a uniform interconnected network with Light microscopy was used to determine the effect of pH on the gel structure. The microscopy images showed an increase in the density and connectivity of fiber networks that was proportional to an increase in pH, as shown in Figure 3. In Figure 3, (A) showed a uniform interconnected network with a concentration of 10 x 10-3 M NaOH (pH ~12.7). Image (B) in Figure 3 used a concentration of 7.5 x 10-3 M NaOH (pH ~12.5) and (C) in Figure 3 had a concentration of 5 x 10-3 M NaOH (pH ~12.3); these images show a jump between where the gel is still able to form a fiber network. From (B) to (C), a loss of stability occurs due to the fibers having less of an interconnected network. Image (D) has a sparser formation with a concentration of 2.5 x 10-3 M NaOH (pH ~12.1). In image (D) a gel was not fully able to form. This is consistent with the hypothesis that the lower concentration of NaOH was unable to create enough fiber networks to make a stable gel.

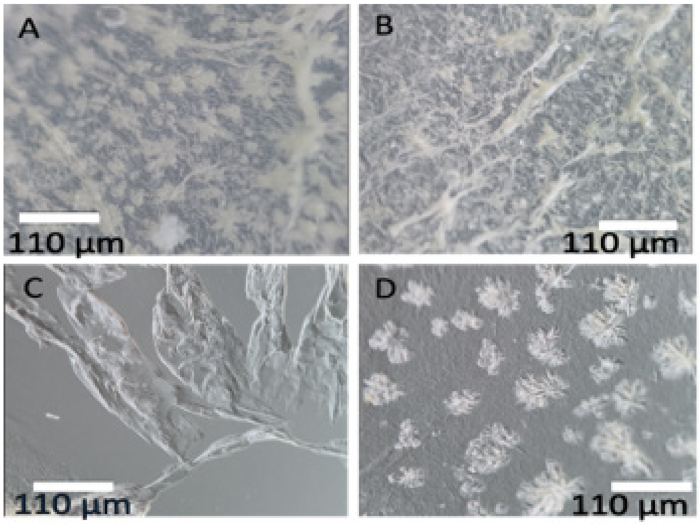

Fig 3. Microscopy images on Fmoc solutions.

Proton nuclear magnetic resonance spectroscopy (H-NMR) was used to better understand the non-covalent interactions in the gel. Along with NaOH, urea is used to help dissolve cellulose in aqueous solutions; therefore, the interactions between urea and Fmoc also need to be considered. H-NMR shows the chemical shifts and hydrogen bonding that occur when the different compounds are mixed together. The H-NMR spectra in Figure 4 indicate that there are interactions between the Fmoc and NaOH, but not Fmoc and urea. Specifically, comparing peaks (a) and (b) for native Fmoc with respect to the other mixtures, it can be seen that these peaks are only suppressed with the addition of NaOH – indicating interactions via hydrogen bonding between the two compounds. Furthermore, it can be seen that a urea peak is present in the Fmoc and urea sample, while it is missing in the Fmoc, NaOH, and urea sample; this indicates that there are hydrogen bonding interactions between the urea and NaOH compounds, but not between urea and Fmoc.

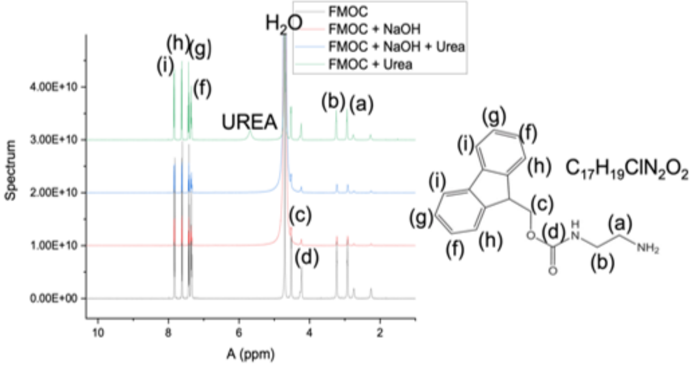

Fig. 4 HNMR Microscopy images on Fmoc solutions.

In summary, there is an optimal range for pH during gelation of Fmoc-based gels that influences the formation of the fiber network needed for gel stability. Additionally, there are time dependent effects on gel stability that relate to gel composition. Lastly, H-NMR spectroscopy indicates hydrogen bonding and physical interconnections between NaOH and Fmoc that can be used during gelation with cellulose solution to tune gel properties. Future work includes characterizations of these Fmoc gels coassembled with cellulose gels as well as mechanical testing to quantify gel stability.

Statement of Research Advisor

Jordan is an excellent researcher and her leadership, enthusiasm, and productivity has helped answer key fundamental research questions about co-assembly of small-molecules with biopolymers like cellulose. She is independently leading this project and her work is critical to solving materials and manufacturing design problems related to cellulose gels and microgels. I am proud of the expertise she has gained in nontrivial analytical techniques like NMR and microscopy and look forward to witnessing her very promising research career.

- Symone Alexander, Department of Chemical Engineering, Samuel Ginn College of Engineering

References

[1] *Cellulose.* American Chemical Society. (n.d.). Retrieved April 3, 2023, from https://www.acs.org/molecule-of-the-week

[2] Croitoriu, A., Nita, L. E., Chiriac, A. P., Rusu, A. G., & Bercea, M. (2021). New physical hydrogels based on co-assembling of FMOC–amino acids. *Gels, 7*(4), 208. https://doi.org/10.3390/gels7040208

[3] Croitoriu, A., Nita, L. E., Rusu, A. G., Ghilan, A., Bercea, M., & Chiriac, A. P. (2022). New Fmoc-amino acids/peptides-based supramolecular gels obtained through co-assembly process: Preparation and characterization. *Polymers, 14*(16), 3354. https://doi.org/10.3390/polym14163354

[4] Dudukovic, N. A., & Zukoski, C. F. (2014). Mechanical properties of self-assembled Fmoc-diphenylalanine molecular gels. *Langmuir, 30*(15), 4493–4500. https://doi.org/10.1021/la500589f

[5] Karunakaran, S. C., Cafferty, B. J., Jain, K. S., Schuster, G. B., & Hud, N. V. (2019). Reversible transformation of a supramolecular hydrogel by redox switching of Methylene Blue—a noncovalent chain stopper. *ACS Omega*, 5(1), 344–349. https://doi.org/10.1021/acsomega.9b02785

[6] Korhonen, O., & Budtova, T. (2019). Gelation of cellulose-naoh solutions in the presence of cellulose fibers. *Carbohydrate Polymers*, 224, 115152. https://doi.org/10.1016/j.carbpol.2019.115152

[7] Li, W., Zhang, L., Ge, X., Xu, B., Zhang, W., Qu, L., Choi, C.-H., Xu, J., Zhang, A., Lee, H., & Weitz, D. A. (2018). Retrieved Jan 27 2020. Microfluidic fabrication of microparticles for biomedical applications. *Chemical Society Reviews*, 47(15), 5646–5683

[8] Lopez-Sanchez, P., Schuster, E., Wang, D., Gidley, M. J., & Strom, A. (2015). Diffusion of macromolecules in self-assembled cellulose/hemicellulose hydrogels. *Soft Matter, 11*(20), 4002–4010. https://doi.org/10.1039/c5sm00103j

[9] Murali, D. M., & Shanmugam, G. (2019). The aromaticity of the phenyl ring imparts thermal stability to a supramolecular hydrogel obtained from low molecular mass compound. *New Journal of Chemistry*, 43(31), 12396–12409. https://doi.org/10.1039/c9nj01781j

[10] Tao, K., Yoskovitz, E., Adler-Abramovich, L.,

& Gazit, E. (2015). Optical property modulation of Fmoc Group by ph-dependent self-assembly. *RSC Advances*, 5(90), 73914–73918. https://doi.org/10.1039/c5ra16412e

[11] Yang, Y., Sha, L., Zhao, H., Guo, Z., Wu, M., & Lu, P. (2023). Recent advances in cellulose microgels: Preparations and functionalized applications. *Advances in Colloid and Interface Science, 311*, 102815. https://doi.org/10.1016/j.cis.2022.102815

Authors Biography

Jordan N. Clemmons is a sophomore-year student pursuing a B.S. degree in Chemical Engineering. She is an undergraduate researcher for the Alexander Research Team.

Dr. Symone Alexander is an Assistant Professor in the Department of Chemical Engineering at Auburn University. During her time as an Eckert Postdoctoral Research Fellow at Georgia Institute of Technology in the Department of Chemical and Biomolecular Engineering, she led investigations on extreme organismic biophysics with a focus on ultra-fast motion in nature in Prof. Saad Bhamla's research group. She earned her Ph.D. in Macromolecular Science and Engineering as an NSF Graduate Research Fellow at Case Western Reserve University, advised by Prof. LaShanda Korley. During her graduate career, she led research on the influence of high molecular weight polymers on self-assembling small molecules and how those networks can be utilized to generate bioinspired, responsive polymer composites. She obtained a B.S. in Chemical Engineering from Howard University in 2013, where she investigated DNA-polymer assemblies utilizing Atomic Force Microscopy under the advisement of Dr. Preethi Chandran and Dr. Joseph Cannon. Dr. Alexander recently served as the elected chair of the 2019 Polymers Gordon Research Seminar (GRS) and actively participates in science communication through via Twitter. She is a recipient of numerous awards and honors, including grad and postdoc fellowships, selection as a 2020 Emerging Leader by Georgia Tech Dept. of Mechanical Engineering, selection as a 2019 American Chemical Society Future Faculty Scholar (ACS PMSE), and being selected as a 2018 Rising Star in Chemical Engineering by the Massachusetts Institute of Technology (MIT).