The Behavior of Fe^{3+} Coordination in Alginate-Catechol Networks

by

Stephanie E. Marzen

Submitted to the Department of Materials Science and Engineering
in Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science

at the

Massachusetts Institute of Technology

February 2015

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ABSTRACT

Mussel byssal threads allow mussels to remain steadfast on ocean rocks despite ocean turbulence, facilitated by the simultaneous elasticity and hardness of the byssus coating. Inspired by the metal-coordination chemistry found in byssus coating, scientists have synthesized an array of self-healing hydrogels with catechol-modified, 4-branched PEG (4cPEG) and various metal ions, primarily Fe^{3+}. While considerable testing has been conducted with 4cPEG, the effects of changing the polymer backbone have not been investigated extensively. Here, alginate was chemically modified with catechol attachments (Alg-C), and metal-coordinated with Fe^{3+} to yield a self-healing network with similar qualities to 4cPEG gels. Rheological measurements indicated that metal-coordination played a dominant role in the bulk mechanics of the network, although the ionic crosslinking caused the gel to act as a solid across all frequencies, in contrast to 4cPEG. In addition, the stiff alginate backbone caused the metal-coordinate bond in itself to act on a longer time scale. Color changes in the Alg-C gel indicated that excess catechol on the backbone was oxidizing. While rheology confirmed the metal-coordination in the Alg-C network, UV-vis absorption measurements provided less certain data. Nonetheless, this study shows that metal-coordination is highly dependent on the polymer backbone, but may still be used in a variety of polymer networks.

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Acknowledgements

I would like to thank all members of my lab group, the Laboratory for Bio-Inspired Interfaces (LBI) for their valuable input and help with instrumentation, especially Scott Grindy, Pangkuan Chen and Qiaochu Li. I would also like to thank Wade Wang for running NMR and GPC samples, Anna Osherov and Tim McClure of the CMSE for UV-vis instrumentation access and assistance, my friends and family for their continuous support, and last but not least, Professor Holten-Andersen for guiding me from the start of my project.

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1 Introduction

1.1 The prevalence of metal-coordination in mussel fibers

Mussel fibers have undergone extensive investigation, because of the unusual ability of the outer coating, known as the cuticle to effectively protect the fiber and withstand strong ocean currents on a flexible substrate.[1] The cuticle was found to contain granules of dense metal-crosslinking, with catechol groups on L-dopa with Fe^{3+}.[2] Such discoveries launched a plethora of work in the area of metal-coordination, including other metals such as vanadium and aluminum.[3]

1.2 Overview of alginate

Alginate is an anionic polysaccharide that contains *cis*-hydroxyl groups and carboxyl groups, opening the doors for a range of chemical modifications.[4]Alginate cross-links electrostatically not only with divalent cations such as calcium, but also with other metals including iron; recently, multiple studies have been done on alginate-iron beads, with calcium included in the mix.[5] Alginate is commonly studied for biomedically applications – although calcium-alginate used to be a commonly considered option,[6] covalently crosslinked, catechol-modified alginate has proven to be a more stable gel that has greater viability in the biomedical field.[7]

Although most metal-coordination studies in mimicry of mussel cuticle chemistry have worked with a 4-branched, catechol-modified form of polyethylene glycol shown in Figure 1 (4cPEG),[8] this study investigates the possibility of using the same metal-coordination chemistry in an alginate-catechol network (Alg-C). Some important differences in molecular structures include the presence of carboxylic acid groups on the Alg-C backbone, the bulkiness of the

backbone due to the saccharide units, the high molecular weight of alginate, and the random placement of catechol along the backbone.

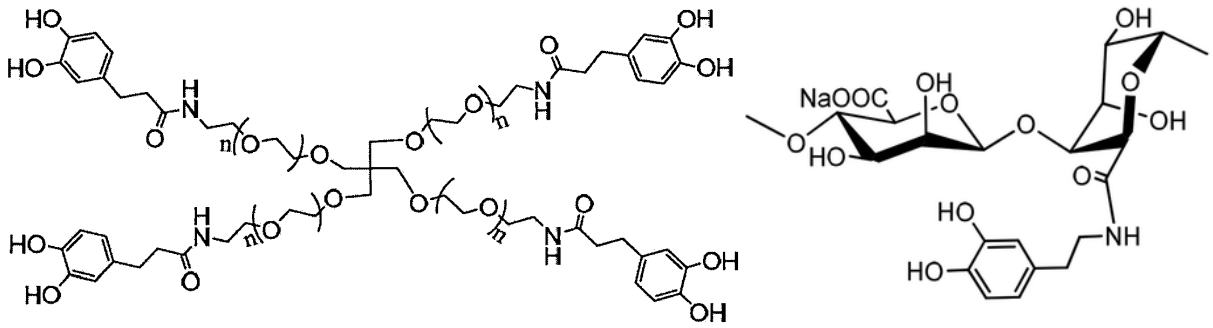

Figure 1. Comparing molecular structures of 4cPEG and Alg-C.

1.3 EDC/NHS reaction

Carbodiimide chemistry is commonly used for modification of carboxylic acid groups.[9] EDC [N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide] and NHS [N-hydroxysuccinimide] together activate the carboxyl acid group and stabilize the intermediates of the reaction to promote the formation of the amide bond. The reaction is conducted in buffer solution,[7,10] to maintain a stable pH during the reaction. EDC operates most efficiently at pH 4-5 and NHS in slightly alkaline solution.

2 Materials and Methods

2.1 Materials

Alginic acid sodium salt was purchased from Sigma Aldrich, with a molecular weight in the general range of 40-80 kDa, according to customer service. Dopamine hydrochloride, sodium periodate, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS), MES hydrate, Iron (III) chloride in hexahydrate form, and sodium hydroxide in pellet form were all purchased from Sigma Aldrich.

2.2 Synthesis of catechol-modified alginate (Alg-C)

Alg-C was synthesized following a procedure similar to that outlined by Kastrup et.al. [10] Three samples of Alg-C were prepared with the aim of conjugating more or less dopamine onto the alginate backbone. For each sample, dopamine hydrochloride was dissolved in 2mL of 0.1M MES, EDC and NHS were dissolved in a separate vial also containing 2mL of 0.1M MES, and the solutions were then mixed with 0.1g of alginate dissolved in 10mL of 0.1 MES buffer for ~80 minutes. The solution was dialyzed using 3.5 kDa snakeskin tubing, 35mm diameter for 60 hours total. The first 12 hours of dialysis were conducted in 500 mL of Milli-Q, ultra-pure water, while the next 48 hours were conducted in 1 L of water. Water replacements were conducted after the first 12 and 36 hours. After dialysis, samples were freeze-dried for at least 48 hours.

All samples were prepared in 0.1M MES buffer, For MES #1 (sample containing the least dopamine), the reagent quantities were 108.1mg of dopamine hydrochloride, 109.28 mg EDC, and 123.78 mg NHS. The reaction vial for MES #2 contained 72.1 mg dopamine hydrochloride, 72.9 mg EDC, and 82.52 mg NHS, and the reaction vial for MES #3 contained 36.0 mg dopamine hydrochloride, 36.4 mg EDC, and 41.26 mg NHS.

2.3 Hydrogel preparation

Alg-C hydrogels were prepared on Parafilm as shown in Figure 2. Although 300 μL total of 1 wt% Alg-C polymer was used in each sample, the slow reaction kinetics of the polymer prevented successful preparation at such a high volume, at least within a reasonable time period. Thus, the synthesis was divided into 100 μL samples of Alg-C solution, to which 3.79 μL of 0.5M $FeCl_3$ and varying amounts of 2.5M NaOH were added. $FeCl_3$ was added in a 1:3 molar ratio of $Fe3^+$: carboxyl acid groups on the alginate backbone. The addition of $FeCl_3$ resulted in a stiff gel of light green color shown in (b), while NaOH addition caused a red color change, and slow relaxation of the gel as shown in (c). The individual, 100 μL gel samples were then combined as shown in (d), and placed underneath a glass vial for 30 minutes to prevent dehydration for the majority of the reaction, and subsequently air-dried for 3 hours to allow for a more solidified and elastic sample. Final polymer weight percentages ranged from 2-4 wt%, based on the final mass of the hydrogel before mechanical testing.

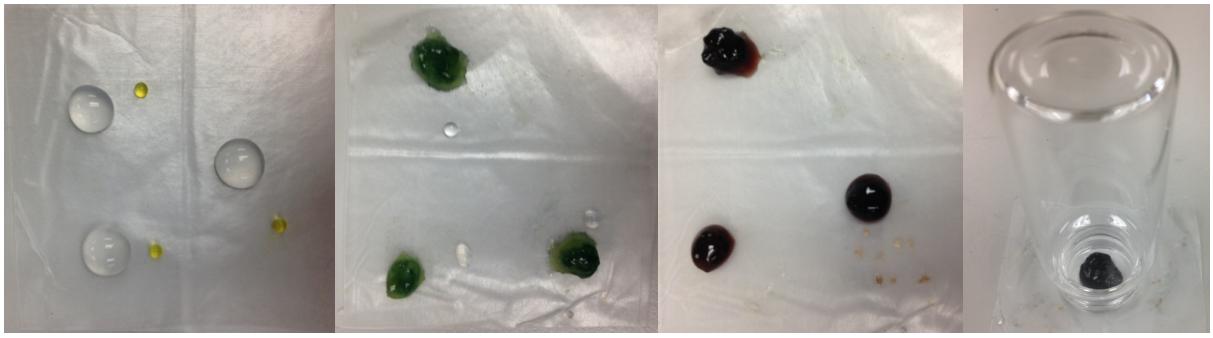

Figure 2. Sample preparation of Alg-C, Fe^{3+}-coordinated gels at high pH.

For targeting a range of pH values, incremental amounts of 2.5M NaOH were added to each 100 μL sample: 0 μL, 1.14 μL, 2.27 μL, 3.41 μL and 4.54 μL yielding approximate pH values of 2, 3, 6, 9, and 10.5. pH measurements were taken using the pH meter, SoilStik from

Spectrum Technologies. Most Alg-C hydrogels were prepared at pH 10.5, unless otherwise specified.

Other hydrogels prepared for characterization purposes include alginate with periodate (Alg-C-periodate), alginate with only iron added (Alg-Fe), alginate with iron at high pH (Alg-Fe-OH), and alginate itself (Alg). Alg-Fe and Alg-Fe-OH were prepared the same way as Alg-C hydrogels, except using 1 wt% alginate instead of 1 wt% Alg-C. Alg-Fe did not contain any sodium hydroxide, while a pH of 10.5 was targeted for Alg-Fe-OH. All above samples, except for Alg-C-periodate were air-dried for 3 hours.

Alg-C-periodate was prepared by adding enough sodium periodate ($NaIO_4$) to a 300 μL sample of 1 wt% Alg-C to create a molar ratio of 2:1 for dopamine: $NaIO_4$. The Alg-C-periodate sample used for UV-vis and rheology tests contained 300 μL of 1 wt% MES #2, to which 16.59 μL of 0.1M $NaIO_4$ was added. The sample was allowed to set for 24 hours under a glass vial to limit dehydration, and subsequently air-dried for one hour. The final percentage of polymer in the Alg-C-periodate gel was 1.3 wt%.

Fe^{3+}-coordinated hydrogels containing 4-branched, polyethylene glycol with catechol pendant groups on each branch (4cPEG) were prepared by adding 13.3 μL of 80 mM $FeCl_3$ to 40 μL of 200 mg/mL4cPEG, then jumping the pH to ~13.1 with the addition of 26.7 μL of 1M NaOH.

2.4 Nuclear magnetic resonance spectroscopy (NMR)

NMR analysis was conducted on the Bruker 400 to confirm the presence of dopamine in Alg-C. The NMR peaks of dopamine were found in Alg-C samples, and matched those reported in literature.[7]

2.5 UV-vis characterization

The Cary 500i UV-vis-NIR dual-beam spectrophotometer in the ASEF-OTC Lab and the UV-Visible-NR Transmission/Reflectance Spectrophotometer of the Metrology Lab in the CMSE were used for UV-vis analysis. Liquid sample analysis was conduced in a quartz cuvette purchased from VWR, of rectangular shape with path length 10 mm and wavelength range of 170-2700nm. Alg-C was dissolved in water for liquid samples, and UV-vis spectra for alginate samples at matching concentrations were manually subtracted. UV-vis solid samples were prepared by squeezing the gel between two microscope slides of soda lime glass, also purchased from VWR.

2.6 Rheology

Rheology was conducted on the Anton Paar, Modular Compact Rheometer (MCR) 302. Frequency sweeps were conducted at a constant strain of 1%, with an angular frequency ranging from 100 to 0.01 rad/s. Amplitude sweeps were also performed to ensure that the applied shear stresses did not extend beyond the viscoelastic range of the hydrogel. Most rheology was completed using the conical plate of 25mm diameter, CP25-1 although the stiffer gel samples did not cover the conical plate, and the parallel plate of 10mm in diameter, PP10 was used instead.

Most rheology was done at 25°C, but some temperature sweeps were performed at temperatures ranging from 20-40°C. The set-up for temperature sweeps is shown in Figure 3: play-doh and mineral oil incased the sample to minimize dehydration as shown in (a) and (b).

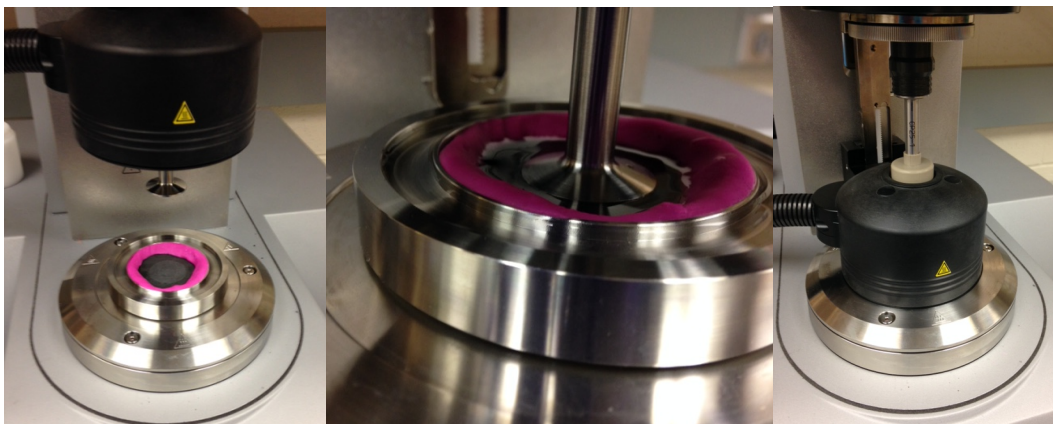

Figure 3. Rheology set-up for temperature sweeps.

3 Results

3.1 Dopamine conjugation

Beer's law was used to calculate the percentage of carboxyl acid groups on the alginate backbone conjugated with dopamine, using results from UV-vis spectroscopy. Figure 4 highlights the dopamine peak at 280nm, at varying concentrations of MES #2.The sample preparation of Alg-C outlined in section 2.2 yielded fairly consistent results, given two sets of samples collected. The conjugation efficiencies for MES #1, #2, and #3 were 12.9%, 17.1% and 20.7% for the first trial and 14.4%, 19.4% and 22.7% for the second trial.

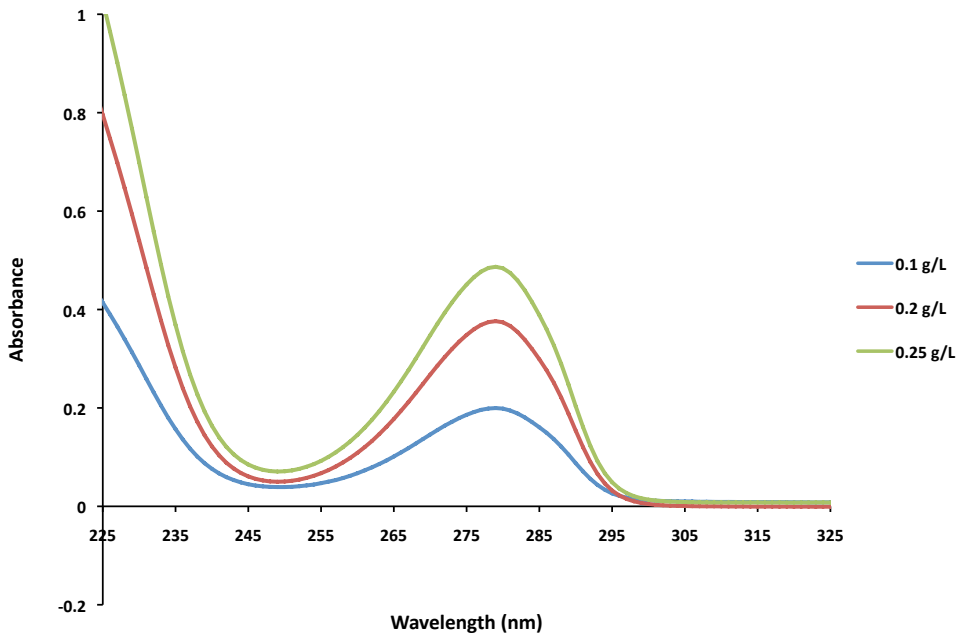

Figure 4. UV-vis spectroscopy of Alg-C, highlighting the dopamine peak at 280nm.

Alg-C hydrogels tested in this report were prepared using the second batch of samples, mostly using MES #2 because it yielded more solidified gels than MES #1, and appeared to contain fewer impurities than MES #3 – the second batch of MES #3 was actually scaled by 3x, which resulted in slightly less homogenous solutions.

3.2 Self-healing capabilities and qualitative observations of mechanical properties

Although rheological data is highly valuable in measuring mechanical properties of soft materials, equally valuable information may be obtained from preparing and working with the samples. The simplest way of determining wither a solid gel is self-healing is to cut it in half, and observe it re-healing, as shown in Figure 5. The particular Alg-C sample shown is MES #1, which is unusually soft; thus, in (a) the hydrogel is in its most relaxed state and has spread out somewhat across the Parafilm surface, but after cutting the gel in half and placing the two halves together as in (b) and (c), the gel is already in a slightly more agitated state, but it nonetheless

heals together as shown in (d) and (e). Alg-C samples MES #2 and MES #3 self-healed in similar fashions, although such gels were stiffer to begin with and thus took slightly longer to self-heal.

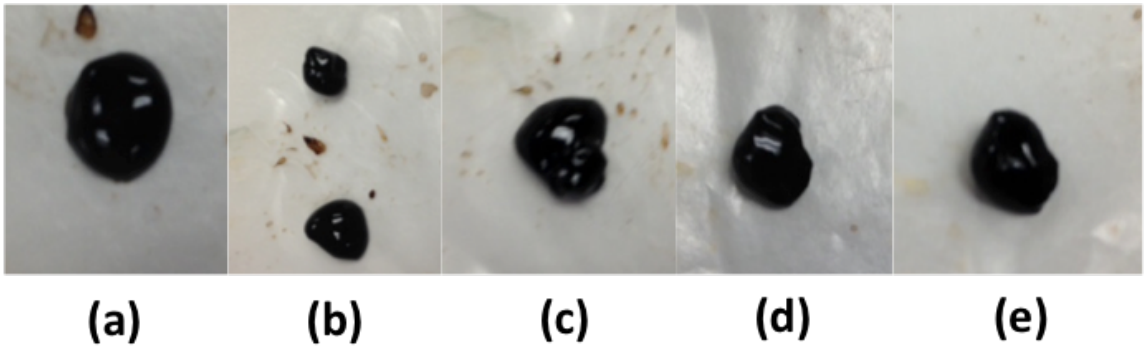

Figure 5. Demonstration of self-healing capabilities for Alg-C hydrogels.

Figure 6 highlights the stark contrasts, as well as surprising similarities observed between hydrogels derived from Alg, Alg-C and 4cPEG. Adding $FeCl_3$ to alginate as shown in (a) results in a stiff, gloppy and heterogeneous network that does not retain water as successfully as most other samples, while Alg-Fe-OH was a surprisingly homogeneous network with similar mechanical properties as the Alg-C hydrogel in (c); no Fe^{3+} appeared to precipitate out of the gel in the form of $Fe(OH)_3$, although such precipitates are readily observed if too much $FeCl_3$ is added in sample preparation. The most surprising difference in mechanical behavior between the Alg-C and 4cPEG gel was the singular ability of 4cPEG gels to extend into fibers. In contrast, Alg-C gels are much stiffer and do not naturally extend into fibers; Alg-C gels also do not retain water nearly as well as 4cPEG gels, most likely because Alg-C gels were prepared at much lower weight percentages (generally 2-4%) than 4cPEG gels, which were 15 wt%.[3] Placed on a hydrophilic surface, Alg-C hydrogels lose some water content.

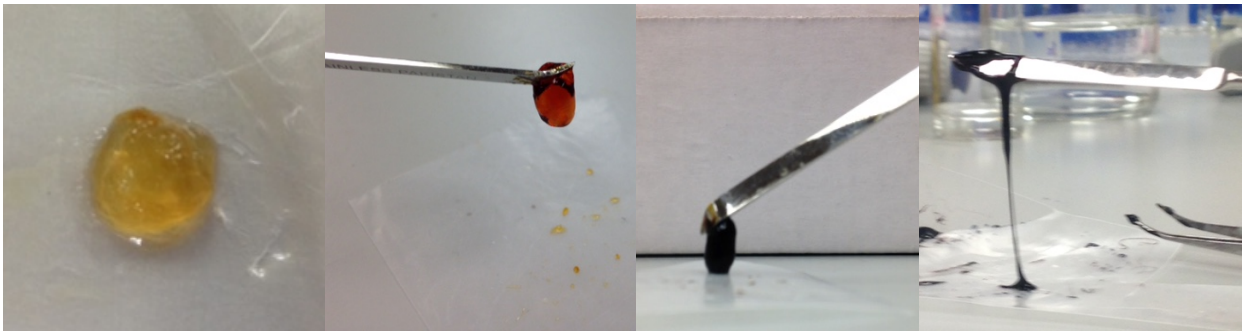

Figure 6. Qualitatively comparing the mechanical behavior of hydrogels: (a) Alg-Fe, (b) Alg-Fe-OH, (c) Alg-C gel, from MES #1 and (d) 4cPEG gel.

3.3 Frequency sweeps

Although the rheological data below provides some insight into the mechanical properties of the hydrogels, comparisons of gel stiffness from storage modulus values must be made carefully, given the broad distribution of polymer wt% in each hydrogel. Although all of the hydrogels were air-dried for the same period of time, and nearly all of the hydrogels contained between 2-4 wt% of polymer, this was enough to cause significant differences in rheological properties. In terms of total mass, most samples weighed approximately 100 mg, but one samples weighed as little as 65 mg. For future testing, samples should be prepared with a targeted mass. Nonetheless, many insights may be obtained from the following frequency sweeps.

For all of the following figures, data is presented on a log-log scale, G' is represented by triangles and G'' by circles. In Figure 7, Alg-C and 4cPEG hydrogels are directly compared. The 4cPEG gel was tested using PP10, the Alg-C MES #2 using CP25-1, and the temperature was 20°C for both sample runs. The frequency sweep for 4cPEG showed the characteristic crossover in storage and loss modulus at frequency ~2.5 rad/s, for a characteristic relaxation time of 0.4s, while the Alg-C sample did not display any crossover, although there was a clear peak at ~0.05

rad/s. For all Alg-C samples, the loss modulus never surpassed the storage modulus in magnitude, indicating that the Alg-C gels have a much stiffer and solid network than the 4cPEG gels. The presence of the loss modulus peak for Alg-C is evidence of passing through a frequency that correlates with a relaxation time in the polymer network – exactly what it correlates with is not entirely clear, but the lack of any peak in loss modulus for other samples indicates that the peak may be correlated with Fe^{3+} coordination in the Alg-C network.

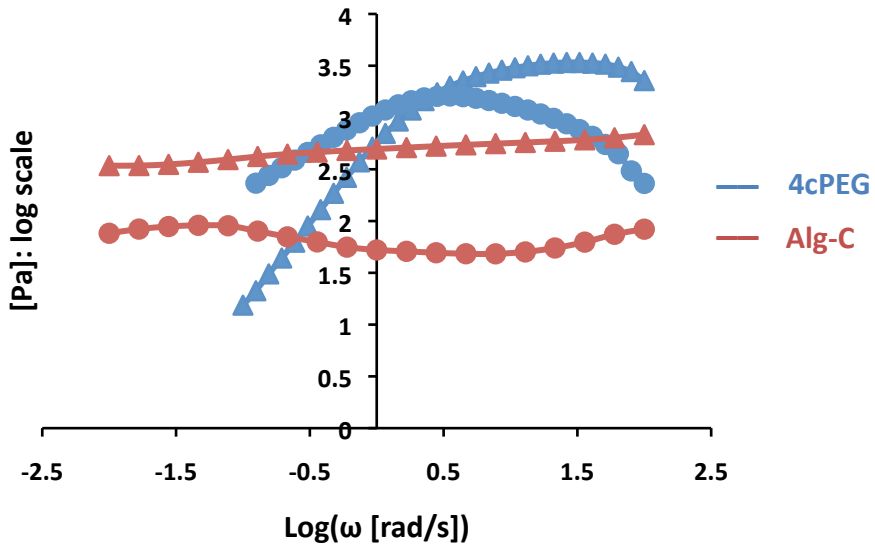

Figure 7. Frequency sweeps of Fe^{3+}-coordinated, catechol hydrogels at pH ~10.5 for Alg-C and pH ~13.1 for 4cPEG.

Figure 8 shows frequency sweeps for Alg-based hydrogels, which do not contain any catechol in the network. Alginate displays a crossover between storage and loss modulus at an unusually high frequency, which is logical given the expectedly low characteristic relaxation time of a viscous fluid in comparison to a gel. Alg-Fe displays a high storage modulus, which is expected of the stiff network containing ionic coordination between Fe^{3+} and COO^-, while G' and G'' for Alg-C hydrogel, Alg-C-periodate and Alg-Fe-OH all lie within the same general range. The Alg-C-periodate graph would likely be shifted upward on the y-axis if it were given

more time to dry – the Alg-C-periodate sample, prepared from MES #2 weighed about 227 mg, whereas most of the samples weighed ~100 mg. However, only the Alg-C hydrogel displays the signature peak in loss modulus. Even Alg-C-periodate, which contains covalently crosslinked catechol, displays no clear peak. Thus, we may be fairly certain that the peak in loss modulus for Alg-C gels appears from the presence of metal-coordination, similar to the 4cPEG gels.

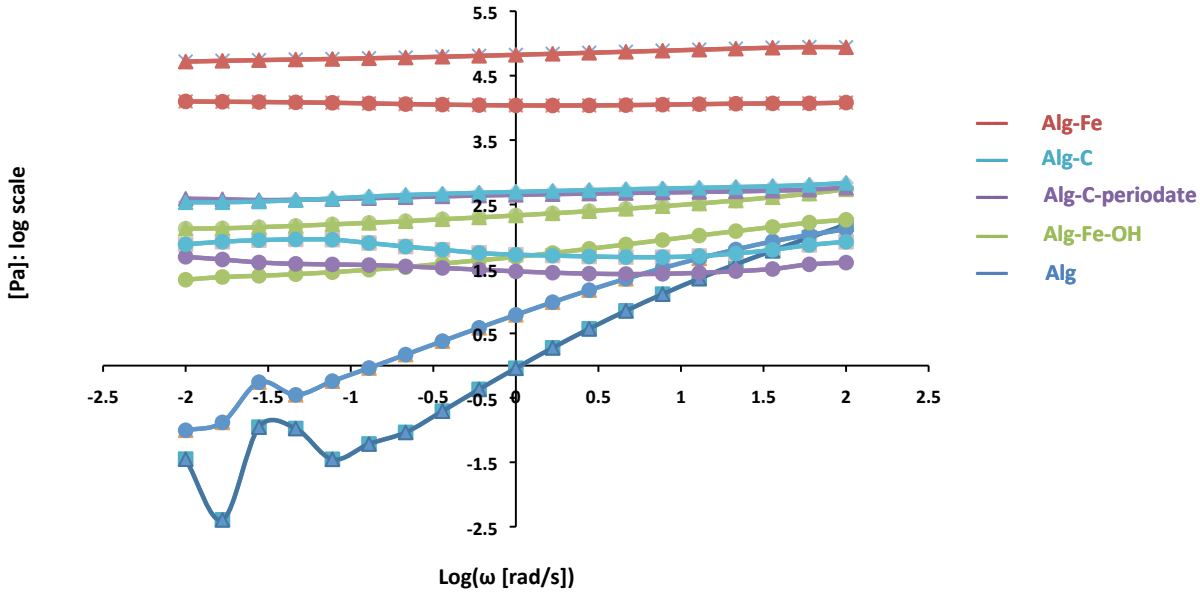

Figure 8. Frequency sweeps of Alg-C hydrogel at pH ~10.5 in comparison to controls: alginate, alginate-Fe, alginate-Fe-OH and Alg-C-periodate.

To further classify the role that metal-coordination plays in Alg-C networks, frequency sweeps were taken at varying temperatures ranging from 20-40°C for MES #2. Figure 9 shows a clear peak shift to the right as the temperature increases from 20-35°C, which is expected given that the peak placement is related to the time-scale on which the metal-coordinate bond behaves, and increased temperature will decrease the associated relaxation time. The activation energy associated with the metal-coordinate bond may be calculated from rheology, although that was not done in this study.

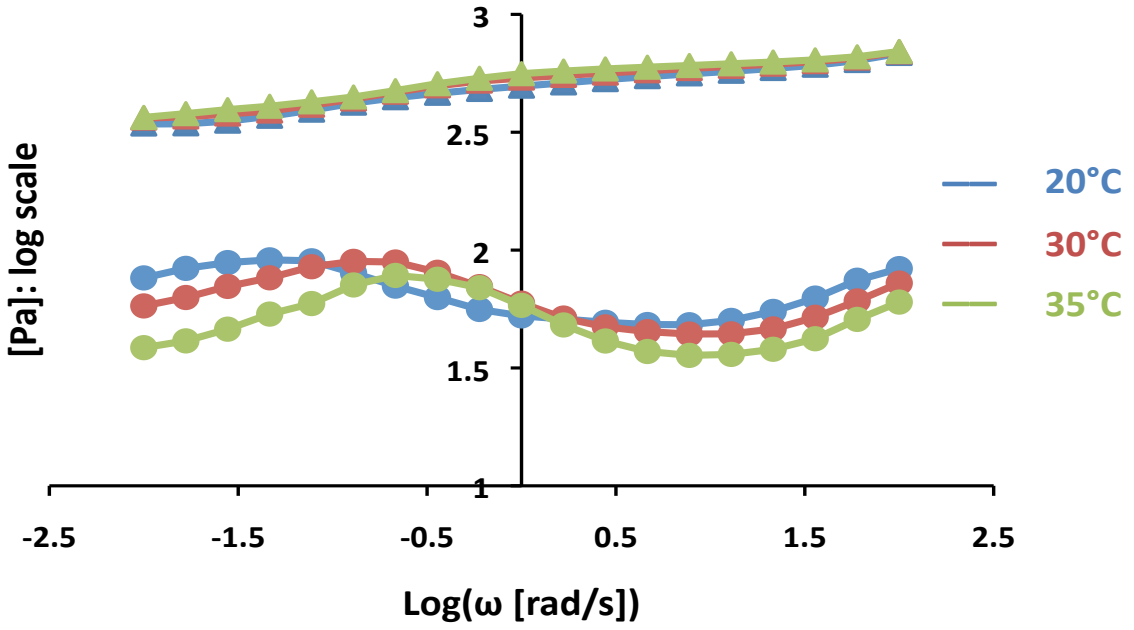

Figure 9. Frequency sweeps for Alg-C at temperatures ranging from 20-35°C.

During sample preparation, varying quantities of NaOH may be added to the Alg-C network, to create hydrogels of pH ranging from 2-10. Although the Alg-C hydrogel at pH 2 is quite stiff, given that Fe^{3+} coordination is not possible at low pH and gelation only results from ionic interactions with the carboxylic acid groups on the alginate backbone, the hydrogel undergoes clear mechanical changes with increasing pH – not only is this an almost certain result of metal-coordination, but also of possible joint formation of an Alg-Fe-OH network within the Alg-C hydrogel. At high pH, the Alg-Fe-OH gel also demonstrated similar mechanical behavior to the metal-coordinated gels, albeit there was no peak in the loss modulus.

Figure 10 shows frequency sweeps at 25°C for Alg-C MES #2 hydrogels at varying pH levels. Differing levels of dehydration, despite equivalent drying times resulted in scattered results and thus no clear trend in storage modulus values, although it appears to generally decrease with increasing pH. However, the peak in loss modulus presumably associated with metal-coordination only appears clearly for pH 10.5. Also worthy of note is the slight increase in

loss modulus for pH 10.5 at low frequency, indicative of significant dehydration even within a single sample run. Such dehydration also makes it more difficult to see the metal-coordination peak.

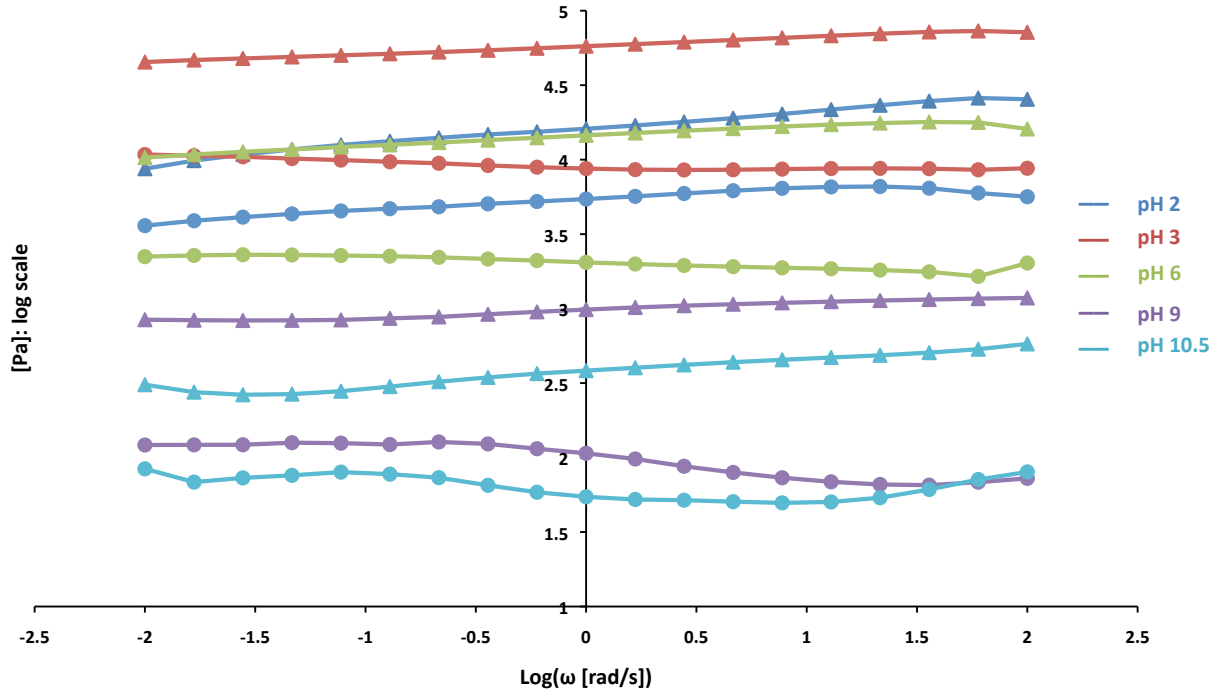

Figure 10. Frequency sweeps of Alg-C hydrogels at varying pH levels. Note the lack of loss moduli peaks for Alg-C samples prepared at pH values lower than 10.5.

Given the different Alg-C samples prepared (MES #1, 2 and 3) with differing amounts of catechol on the alginate backbone, we may examine the effects of increasing dopamine in the network on rheological properties. MES #1 contained ~14% dopamine conjugated, MES #2 ~19% and MES #3 ~23% dopamine conjugation. Alg-Fe-OH was used as the 0% sample. Unsurprisingly, Figure 11 shows that the effects of Fe^{3+} coordination are more pronounced as you increase dopamine conjugation. However, it should also be noted that the 14% Alg-C hydrogel (MES #1) was unusually stiff, because it dried out much more quickly than the other Alg-C gels. Ironically, the most likely reason for it drying more quickly is because it was initially a much more fluid gel, less capable of water retention and more spread out on the Parafilm surface during the air-drying stage, creating a greater surface area for water to escape.

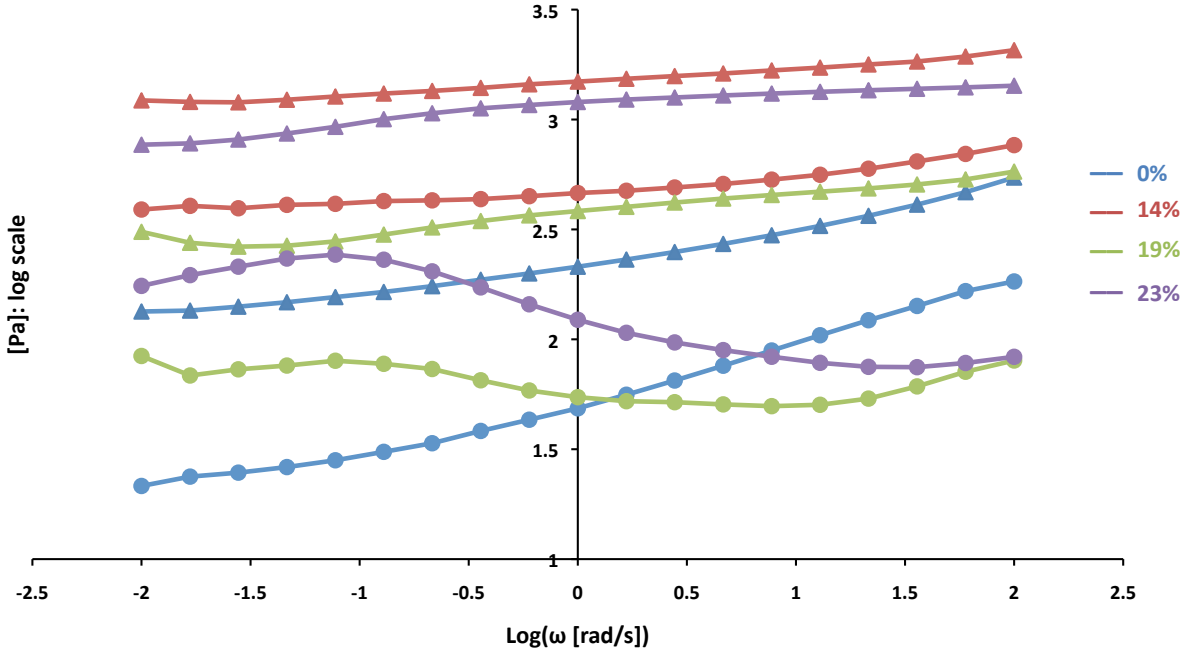

Figure 11. Frequency sweeps of Alg-C hydrogels, with varying dopamine conjugation on the alginate backbone.

3.4 Investigation of dopamine oxidation in Alg-C hydrogels

A purely metal-coordinated hydrogel, if you leave it in water will yield a homogeneous solution, whereas a gel that contains covalent crosslinks will swell, but not dissolve in water. This particular result is highlighted with 4cPEG in metal-coordinated and covalently coordinated form via sodium periodate in Holten-Andersen et. al, where the 4cPEG gel forms a mostly homogeneous solution after 1 hour in EDTA buffer, and the process goes to completion to form a red solution.[3] Interestingly, recent results have shown that adding enough $FeCl_3$ at low pH can promote catechol oxidation, and thus induces some covalent crosslinking via polymerization.[4] Nonethless, the extent of covalent crosslinking was minimal for the 4cPEG gel synthesis, and the gel homogenizes quickly in solution. In contrast, the Alg-C gel left in solution takes at least 36 hours to fully homogenize in water, as shown in Figure 12. Furthermore, the gel placed in

solution was only 100 μL of MES #2, and unlike the other Alg-C gels tested, it was not air-dried. Had the gel been synthesized from a 300 μL sample, and air-dried to increase the polymer wt% from 1 to about 3%, it would have taken significantly longer for the polymer to dissolve in solution, although the dissolution rate may have been reduced to some extent if EDTA were used instead of water, due to EDTA's chelating properties.

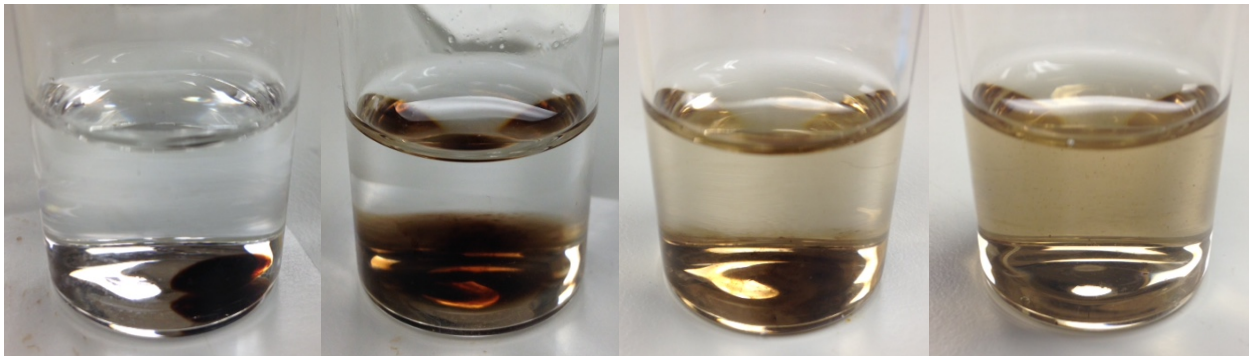

Figure 12. Leaving the Alg-C gel in water overnight, the clear solution formed after 36 hours indicates that minimal, if any covalent crosslinking is present.

The gel's retarded relaxation properties may result from many factors, not limited to ionic interactions, and increased chain entanglement resulting from high molecular weight and a bulky polysaccharide backbone. Although the formation of a homogeneous solution confirms that covalent cross-linking is not affecting the bulk mechanics of the Alg-C gel, nonetheless dopamine oxidation is definitely causing significant color changes, at the minimum. Given that dopamine oxidizes quickly at high pH, this result would not be unexpected. The standard color changes associated with the 4cPEG gel are green, blue/purple, and red with increasing pH.[3] The solution formed in Figure 12 is not the standard red solution characteristic of 4cPEG gels, but a light brown color. UV-vis spectroscopy studies were carried out to investigate the possibility of dopamine oxidation in Alg-C gels.

Figure 13 shows the sample preparation carried out for a side experiment. An excess quantity of Alg-C solution was used in the preparation of an Alg-C gel, because the desired sample for testing was not the gel itself, but rather the solution surrounding it. In (a) 5 μL of 0.5M $FeCl_3$ is being added to 1.5 mL of 1 wt% Alg-C (MES #1), forming the characteristic green/blue gel found at low pH in (b). It is important to note that in (b), the solution surrounding the blue gel contains minimal Fe^{3+}, because Fe^{3+} interacts ionically with carboxyl acid groups on the alginate backbone, forming a stiff gel. Thus, the addition of 50 μL of sodium hydroxide causing a red solution to form in (c) cannot be caused primarily by tris-coordination, because no significant amount of iron is present in the solution. Furthermore, adding sodium hydroxide to alginate does not result in any color change, but adding sodium hydroxide to Alg-C solution does cause the solution to turn red. Thus, the only plausible explanation for the instantaneous red color change of the Alg-C solution is dopamine oxidation on the Alg-C backbone.

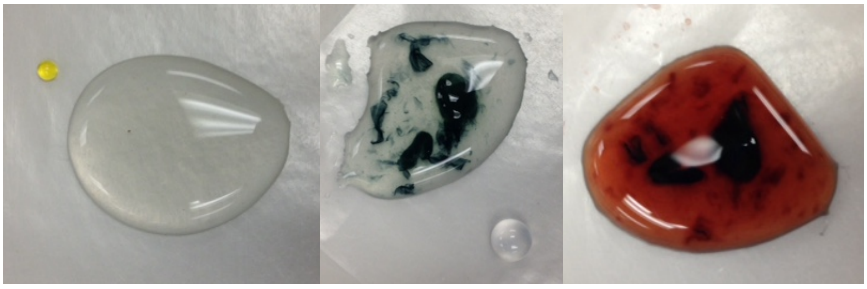

Figure 13. Preparing UV-vis gel solution from Alg-C preparation, testing for dopamine oxidation.

The Alg-C gel solution from Figure 13 was collected for UV-vis testing, and tracked over a period of 4 hours. Approximately 1.7 mL of water was added to the liquid sample to fill the cuvette. Parts (a) through (c) show the darkening from red to brown over time, while (d) is a dilute solution of (c) for better absorption measurements, and (e) is simply dopamine dissolved in water, with sodium hydroxide added to increase the pH.

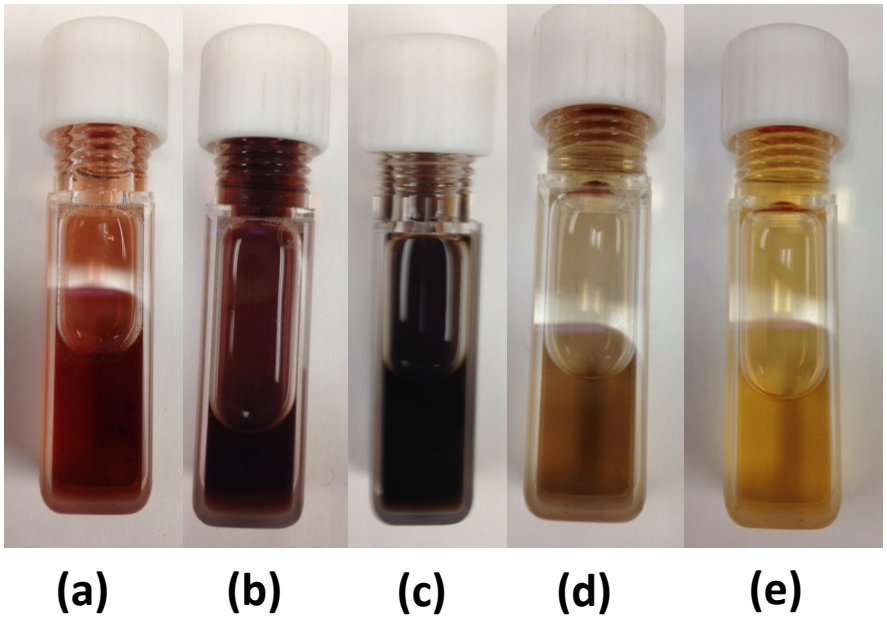

Figure 14. The immediate color formed in solution from Figure 12 is shown in (a), after 30 minutes in (b), and 4 hours in (c). The diluted form of (c) is shown in (d), while dopamine+NaOH is shown in (e).

The UV-vis results from the Alg-C gel solution samples in Figure 14 did not yield conclusive results. Figure 15 shows the absorption spectra for the solutions tested – over time, the peak present at ~450nm, which is associated with a deep red color, disappears after several hours and no clear peak is present for the brown-colored samples. However, the solution containing only dopamine and sodium hydroxide displayed a small peak at 425nm. Such peaks do not match precisely with anything in literature, although the oxidation of catechol to *o*-quinone absorbs at ~400nm,[11] and a study of dopamine oxidation conducted by Burzio et. al obtained similar results, with peaks at 425nm and 462nm.[12] Such peaks found in the 410-460nm rnage were described as not matching to any well known oxidative products of dopamine, but could possibly result from dicatechol formation, which absorbs at 420nm.[12] Surprisingly, this could indicate that some covalent crosslinking is occurring, even if it does not strongly affect the bulk mechanical properties.

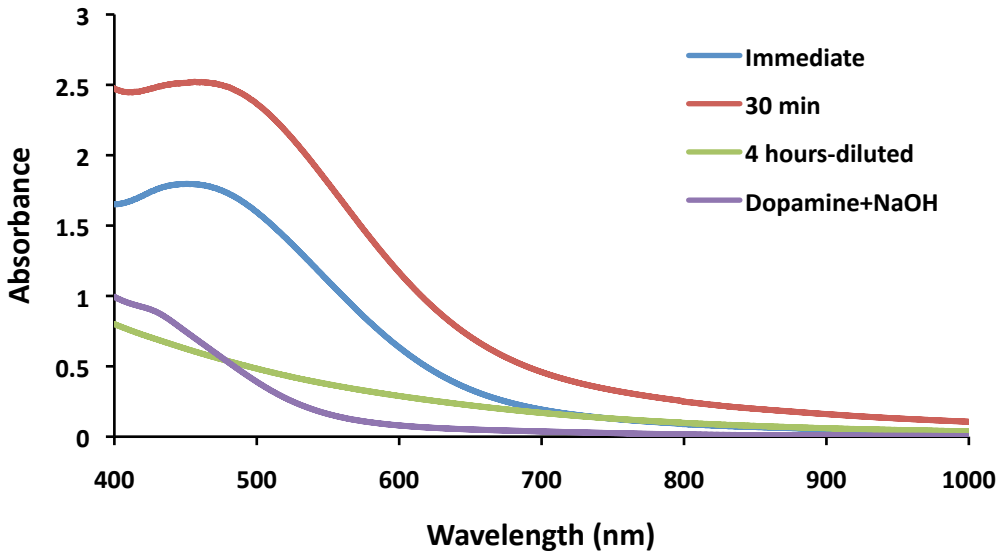

Figure 15. UV-vis spectra for excess Alg-C gel solution, tracked over time for color changes. Peaks are 451nm for the solution tested immediately, 456nm for the sample tested after 30min, 425nm for the dopamine solution at high pH, and no peak is present for the solution prepared after 4 hours.

Unsurprisingly, the Alg-C-periodate sample displays almost identical color changes over time to the Alg-C metal-coordinated samples, displayed in Figure 16. The initial solution is of a yellow color, but within 90 minutes it already forms the red solution characteristic of dopamine oxidation. By the time the solution gelates, it is already a deep brown color. The color changes in Alg-C-periodate provide further evidence that the dominant color changes in the Alg-C network result from dopamine oxidation, and not metal-coordination, because Alg-C-periodate does not have any Fe^{3+} coordination, but still the yellow color initially present completely disappears. The 4cPEG-periodate gel, however, maintains its yellow color for a longer period of time, but does turn slightly brown after sitting overnight.[3] Nonetheless, the dark brown color change in Alg-C gels is certainly caused by dopamine oxidation, and it certainly happens to a greater extent in the Alg-C network than in the 4cPEG network (if it occurs in 4cPEG at all).

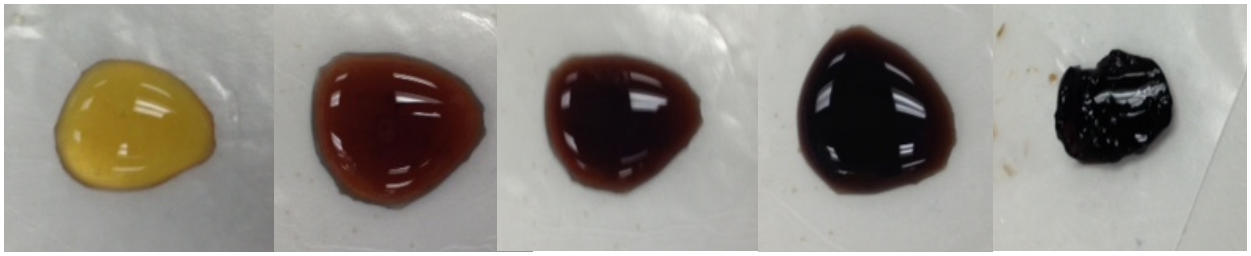

Figure 16. Tracking color changes of Alg-C-periodate gel over time.

The most plausible reason for the large extent of catechol oxidation in Alg-C gels in contrast to 4cPEG, is that the 4cPEG network is much more controlled than the Alg-C network. In Alg-C, dopamine is randomly scattered along the alginate backbone, and it would be physically impossible for the chains to entangle themselves perfectly in order to accommodate metal-coordination for every dopamine. The dopamine content is also much higher in the Alg-C network, conjugated on ~15-20% of each monomer unit. Thus, the Alg-C network is more prone to dopamine oxidation than the 4cPEG network.

In order to confirm the presence of Fe^{3+} coordination in the Alg-C network, UV-vis studies were conducted on the solid Alg-C gel samples at varying pH levels, as well as Alg-Fe-OH and Alg-C-periodate. Most Alg-C samples were only given 30 minutes to homogenize, in order to minimize the effects of dopamine oxidation on UV-vis testing; however, this also resulted in an unusual lack of homogeneity. The UV-vis samples are shown in Figure 17, with Alg-C gels prepared using . Alg-Fe-OH forms a surprisingly homogenous network, which is surprising given that excessive sodium hydroxide would be expected to precipitate iron (III) from the ionic network and form $Fe(OH)_3$. If an Alg-Fe-OH is prepared with excess $FeCl_3$, small particles that are presumably $Fe(OH)_3$ precipitate out of the gel; however, if just enough iron is added to chelate the alginate network, then a homogenous gel forms at high pH. Perhaps the

environment of the alginate network, such as the presence of vicinal hydroxyl groups helps protect the network, or the precipitates are so small that they are not visible to the naked eye.

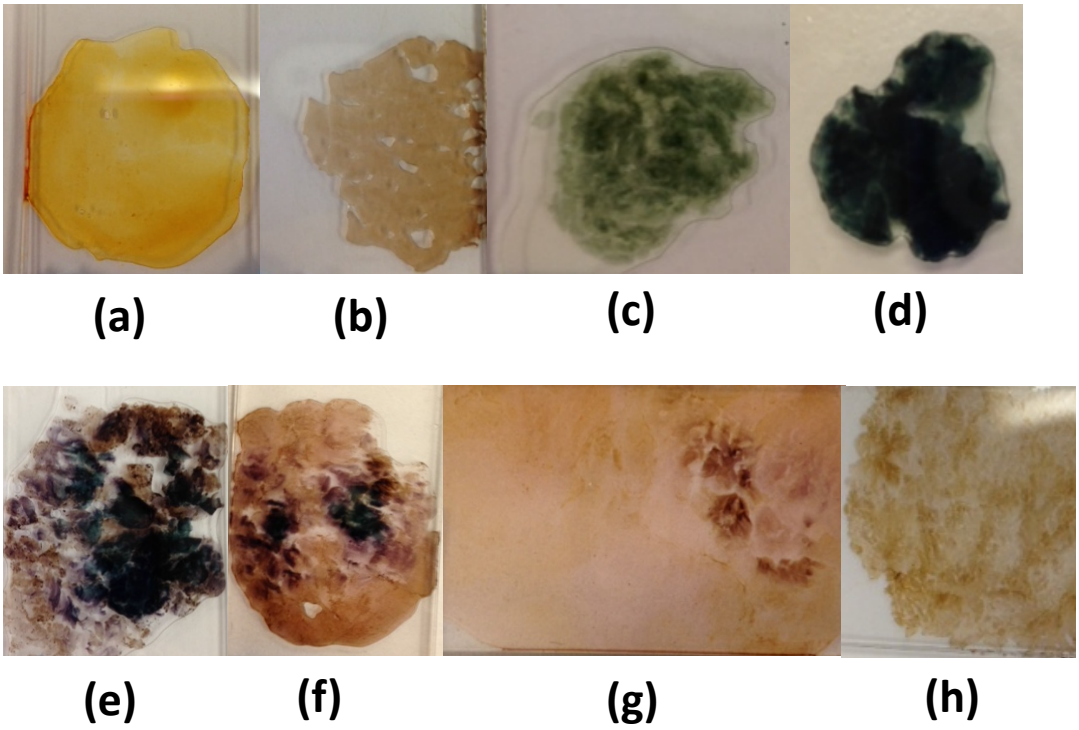

Figure 17. UV-vis gel samples, pressed between two glass slides. (a) Alg-Fe-OH (b) Alg-C-periodate (c) Alg-C gel, pH 2 (d) Alg-C gel, pH 3 (e) Alg-C gel, pH 6 (f) Alg-C gel, pH 9 (g) Alg-C gel, pH 10.5 (h) Alg-C gel, pH 10.5 after several hours.

Sample (b) is represented by Alg-C-periodate, which displays the characteristic brown color associated with dopamine oxidation. Both Alg-Fe-OH and Alg-C-periodate did not display any peaks in the UV-vis absorption spectra, only a general increase in absorption at lower wavelengths approaching 400nm. Thus, we cannot draw any comparisons between the UV-vis spectra for Alg-C hydrogels and control samples.

Samples (c) through (g) represent the Alg-C gels at increasing pH levels, while (h) is an Alg-C sample at pH 10.5 that was left to air-dry for several hours. As expected, dopamine oxidation is clearly present in (h), given that tris-coordination does not result in a brown color, and sample (h) also did not display any clear peaks. Samples (c) through (g), however, did

absorb at specific wavelengths, shown in Figure 18, albeit not at the expected wavelengths from literature.

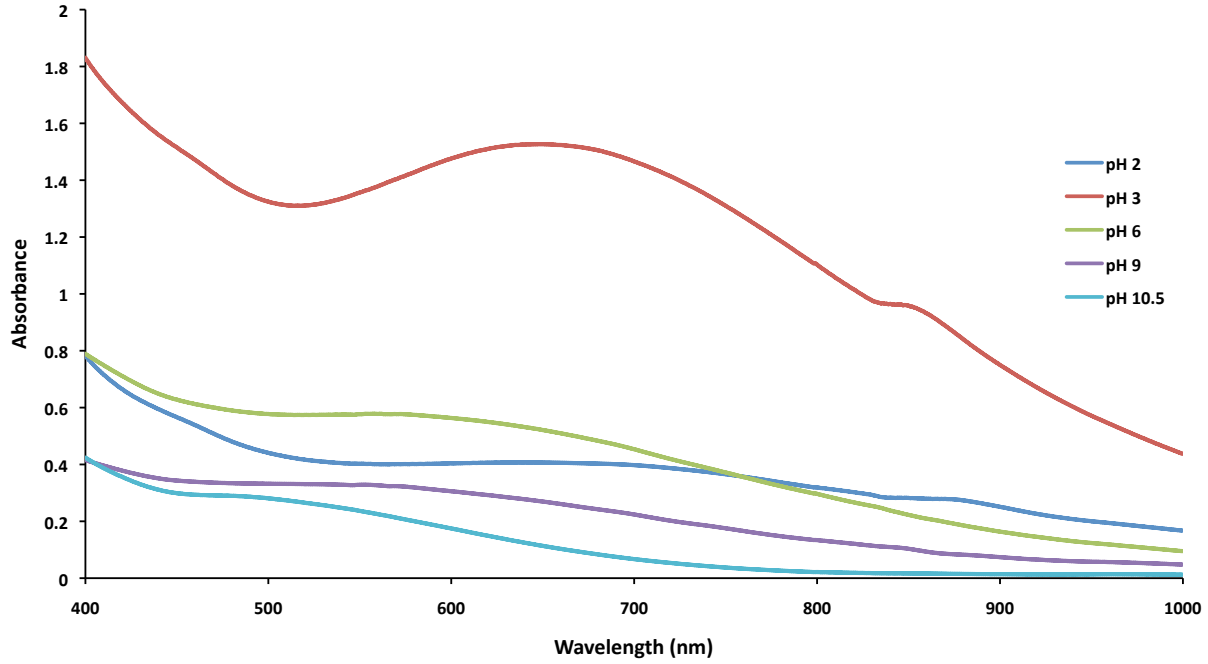

Figure 18. UV-vis gels for Alg-C. Peaks for pH 2 are at 637nm (plateau/peak), 648 (clear peak) for pH 3, 558 plateau/peak for pH 6, 501nm for pH 9, 465nm for pH 10.5.

The absorption peaks shifted to lower wavelengths with increasing pH. At pH 2, there was a slight peak at 637nm, a clear peak at 648nm for pH 3, slight peaks that plateaued somewhat at 558nm and 501nm for pH 6 and pH 9, respectively, and slightly more definite peak at 465nm for pH 10.5. Although the color changes and associated UV-vis data for Alg-C gels at varying pH levels is interesting, it is also difficult to interpret because it does not closely match the values for 4cPEG reported in literature. While the general color transition of green, blue and red with increasing pH is evident in both Alg-C and 4cPEG, 4cPEG gels have clear absorptive peaks at 759nm, 575nm, and 492nm for mono, bis, and tris-coordination as the pH increases.[3] Interestingly, the 4cPEG gels also absorb at lower wavelengths with increasing pH.

The close proximity of the tris-coordination peak in 4cPEG to the absorptive peak for Alg-C at pH 10.5 (492nm v. 465nm), combined with the consistent trends in wavelength

absorption and pH level indicate that metal-coordination may be present in Alg-C gels, but it is certainly not clear evidence of the fact. The possible distortion of the absorptive peaks may result from the increased presence of dopamine oxidative products in the Alg-C gels, the ionic interactions that are also occurring in the network, as well as the vastly different environment of Alg-C compared to 4cPEG. In fact, the Alg-C gels at low pH are definitely a lighter green than the 4cPEG gels, because you can actually see the hints of yellow resulting from Fe^{3+}-COO^{-} coordination. The peaks at higher pH values may not match as well because the peaks associated with dopamine oxidation products are generally found in the low 400nm range. Thus, we cannot expect 4cPEG and Alg-C gels to absorb at exactly the same wavelengths at low or high pH values.

4 Conclusions

This study opened up the possibilities for employing metal-coordination in catechol-modified alginate (Alg-C), when previous studies have generally used catechol-modified 4-branched polyethylene glycol (4cPEG). While the data obtained from spectroscopy and rheology of 4cPEG gels was quite clear and repeatable, due primarily to the simplicity of the network, sample preparation and data analysis of the Alg-C network became more complicated – not only does alginate have a much higher molecular weight than 4cPEG, and consist of a stiffer backbone due to the saccharide units, but it also contains carboxyl acid groups on each unit. Although alginate was specifically chosen because it contains carboxylic acid groups, allowing for simple catechol-modification of the backbone, it also results in Alg-C gels that contain not only metal-coordinate bonds with catechol, but also ionic bonds with carboxylic acid. Because no covalent crosslinking was detected in the network, we may be fairly certain that the unusual

stiffness of the Alg-C gel, especially given the low wt% of polymer in each sample, may be attributed to the additional presence of ionic bonding and high viscosity of the original solution.

Although most evidence points to the presence of metal-coordinate bonds in the Alg-C network: the color changes with increased pH, the self-healing properties of the network, and the tangible similarities to the 4cPEG gel, rheology and UV-vis spectroscopy was employed to provide further evidence that metal-coordination may play a similar role in networks other 4cPEG. Frequency sweeps for Alg-C gels at high pH showed a clear peak in loss modulus at 0.05 rad/s, which shifted with increasing temperature, similar to the peak in 4cPEG. Although the placement of the loss modulus peak was different for the two polymers, we may be fairly certain that both are associated purely with metal-coordination, since the only samples that displayed any peak at all were Alg-C gels at high pH. Furthermore, the displacement of the loss modulus peak may be attributed to the fact that the stiff alginate backbone will have a much less flexible metal-coordinate bond associated with it, thus resulting in a longer relaxation time. The lack of crossover between the storage and loss modulus for Alg-C is indicative of unusual stiffness in the network, almost certainly a result of the ionic crosslinking. A general decrease in stiffness was observed for Alg-C with increasing pH, because some Fe^{3+} that was initially ionically coordinated with carboxylic acid groups switched over to binding with catechol groups in metal-coordinate bonds, which display more flexible properties.

The results from UV-vis spectroscopy were also surprisingly complex for Alg-C. Although the color changes of Alg-C gels from low to high pH appeared similar to that of 4cPEG gels, UV-vis absorption data revealed that the peaks were not quite the same. Furthermore, the color of the Alg-C gels at high pH changed from red to brown within a few hours, which was determined to result directly from dopamine oxidation occurring at high pH. In

fact, the initial products of dopamine oxidation appear red, and slowly transition to brown over time, making it unusually difficult to determine whether or not the red color achieved in Alg-C gels at high pH is a result of tris-coordination, dopamine oxidation or both.

In essence, the main complications associated with the Alg-C network: a stiff backbone, increased chain entanglement resulting in slowed kinetics, presence of carboxyl acid groups on the alginate backbone, and uncontrolled placement of catechol modifications along the backbone result in a metal-coordinated gel that displays some similarities in spectroscopic and bulk mechanical properties to the 4cPEG gel, but also important differences and complications that are difficult to control. Nonetheless, metal-coordination added critical properties to the alginate-catechol network, including self-healing, thus showing that metal-coordination can be successfully employed in more complex networks than star-branched polyethylene glycol.

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