Engineering mechanical dissipation in solid poly(ethylene glycol) hydrogels with bio-inspired metal-coordinate crosslinks

by

Robert Learsch

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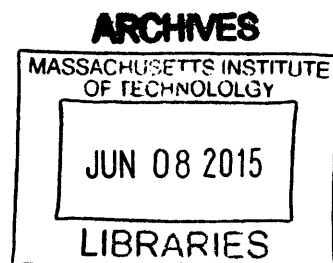Thesis Advisor

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Abstract

Growing evidence supports that the unique mechanical behavior of mussel byssal threads, such
as high toughness and self-healing, rely on an intricate balance of permanent covalent and
reversible metal coordination bonds. Inspired by this material crosslink chemistry balance, we
synthesized polyethylene glycol (PEG) hydrogels with two crosslinked networks; a primary
permanent network composed of covalently crosslinked 4-arm PEG and a secondary network
composed of 4-arm PEG functionalized with histidine on each arm. The histidine decorated PEG
forms a mechanically reversible network via metal ion coordinated crosslinks. Using rheometry,
we study the contribution of the metal-coordinate network to the bulk gels mechanics and find
that we can control both the amplitude and the frequency of peak mechanical dissipation with the
histidine: metal ion ratio and the choice of metal ion, respectively. Furthermore, we can control
the mechanical contribution of metal coordinate bonds by changes in pH. These simple
bio-inspired gels promise to serve as a new model system for further study of opto-mechanical
coupling of metal-coordinate soft materials.

Thesis Advisor: Niels Holten-Andersen

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1. Introduction

Mussels attach themselves to rocks near the shore of the Pacific Ocean, where they are subject to repeated mechanical stress from waves.
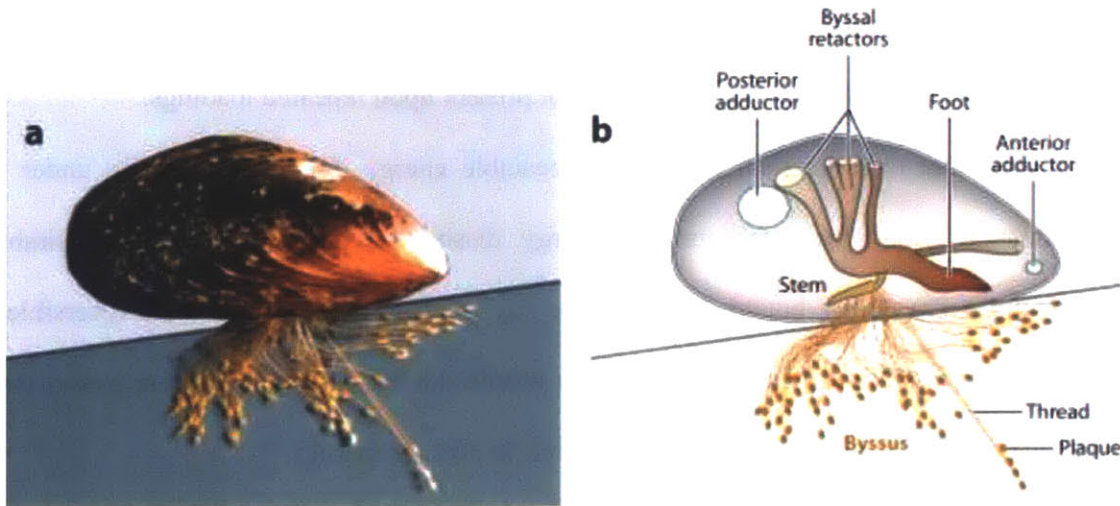
Figure 1.1: a) Adult mussel (5 cm in length) with threads, byssus, attaching to a mica surface. b) Half-shell schematic showing byssus tipped with adhesive plaques. The foot is connected to the stem, which the threads join into[1].

The waves impart large amounts of mechanical energy into the mussels yet they are able to remain firmly attached by secreting proteinaceous fibers called *byssi*. These organic fibers are devoid of cells, and they are simply attached to both the rock and the mussel and able to withstand the repeated stress caused by the incoming waves. Recent discoveries in marine biology have revealed a key component in these fibers that provide energy dissipation in the form of reversible chemical bonds at the interface between the plaque and the thread[2]. The fibers contain metal coordinated bonds: "tris" complexes formed between three (3,4 dihydroxyphenylalanine) (DOPA) ligands and Fe^{3+} ions (see Figure 2.1). These chemical bonds occur underwater at ambient temperature and pressure conditions, and reform spontaneously after a few hours.

The mussel threads unique material properties have not been fully reproduced by synthetic, man-made materials. Dissipative, tunable polymer systems have been synthesized but remain liquid at ambient conditions; solid double network gels show some toughness but rely on breaking covalent bonds to dissipate energy[3]. Because the covalent bonds do not reform, the energy dissipation in this double network is not present upon repeated loadings.

A solid that exhibits such large and repeatable energy dissipation, formed under benign experimental conditions, with tunable energy dissipation properties is thus desirable. By combining a covalently bound poly(ethylene glycol) (PEG) network with a reversible metal coordinated crosslink network, we sought to mimic the basic structure of the byssal thread to investigate the underpinnings of its desirable mechanical properties.

2. Background

Introduction to rheology

Rheology is a powerful tool to measure the mechanical properties of soft materials. It applies a small-amplitude oscillatory shear (SAOS) which can be described as:

$$\varepsilon(t) = \varepsilon_o \sin(\omega t) \tag{1}$$

Where ε_o is the strain amplitude. The stress response is phase-shifted by δ from the driving strain, such that it takes the form of:

$$\sigma(t) = \sigma_o \sin(\omega t + \delta) \tag{2}$$

where δ represents the phase shift angle. It is possible to decompose the stress response into the in-phase and out-of-phase components by applying the trigonometric identity *sin(u+v) = sin(u)cos(v) + cos(u)sin(v)*. Through this identity, we see:

$$\sigma(t) = \sigma_o[\cos(\delta)\,sin(\omega t) + \sin(\delta)\cos(\omega t)] \tag{3}$$

This can be simplified further by defining the coefficients which depend solely on δ, a material property, as the storage and loss moduli (G' and G'', respectively).

$$\sigma(t) = \varepsilon_o[G'(\omega)(\omega t)\,sin(\omega t) + G''(\omega)\cos(\omega t)] \tag{4}$$

$$G' = \sigma_o\cos(\delta), G'' = \sigma_o\sin(\delta)$$

If the stress response is perfectly in-phase with the input strain, $\delta = 0$, G'' = 0, and the material is perfectly elastic. If the stress response is entirely out-of-phase with the input strain, $\delta = \pi/2$, G' = 0, and the material is perfectly viscous. G' and G'' quantify the "solid-like" and "fluid-like" properties of a material and the relative magnitude of G' and G'' is called the "loss tangent,"

which is defined as $G''/G' = \tan(\delta)$. When $tan(\delta) > 1$, the material is fluid-like, and if $tan(\delta) < 1$, the material is solid-like. This value is highly dependent on frequency, ω. In many soft materials, there is an easily attainable crossover frequency, ω_c where $\tan(\delta)$, at $\omega < \omega_c$, is > 1 and $\tan(\delta)$, at $\omega > \omega_c$, < 1. The inverse of the crossover frequency is the characteristic relaxation time, $\tau = 1/\omega_c$.

A common method of oscillatory shear experiment is the "frequency sweep," where the moduli are measured over several orders of magnitude of frequency at a constant strain amplitude.

Inspiration from the mussel thread

To understand and utilize this bonding chemistry, Holten-Andersen *et al* decorated the DOPA molecule onto 4 arm poly(ethylene glycol) (4PEG) to form 4PEG-DOPA[2]. When iron was added and the gels were exposed to correct pH conditions, the 4PEG-DOPA polymers formed a crosslinked gel that exhibited the reversible and repeatable energy dissipative behavior of the mussel threads in a simple mimic. However, this polymer network is liquid at room temperature and could not be used as a dissipative solid in a way similar to what researchers believe the mussel does.
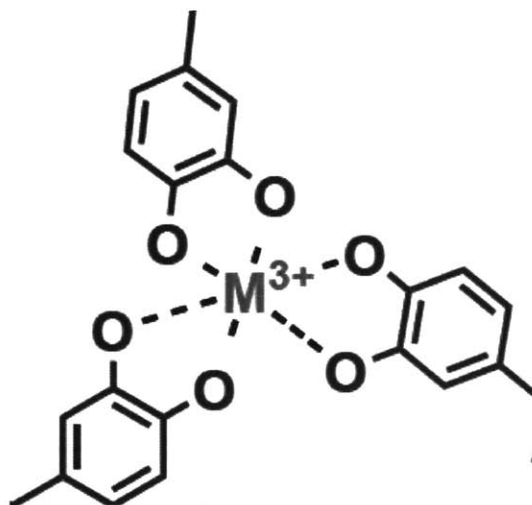
Figure 2.1: Tris complex formed by three DOPA molecules and a metal ion. These structures form spontaneously at high pH and have high bonding energy[4].

The DOPA-metal bonds are modular; they form between DOPA and a trivalent metal ion. Each of the different DOPA-metal complexes possesses different relaxation time – or resonant frequency of the bond – at which the dissipation is most active. By changing the metal ion from Fe^{3+} to Al^{3+}, it is possible to shift the strain rate at which the polymer network dissipates energy most readily over orders of magnitude. Researchers believe we can attribute this change to the difference in ligand exchange kinetics between DOPA-Al and DOPA-Fe complexes[2]. The dissipative properties of mussel threads is shown in Figure 2.2.
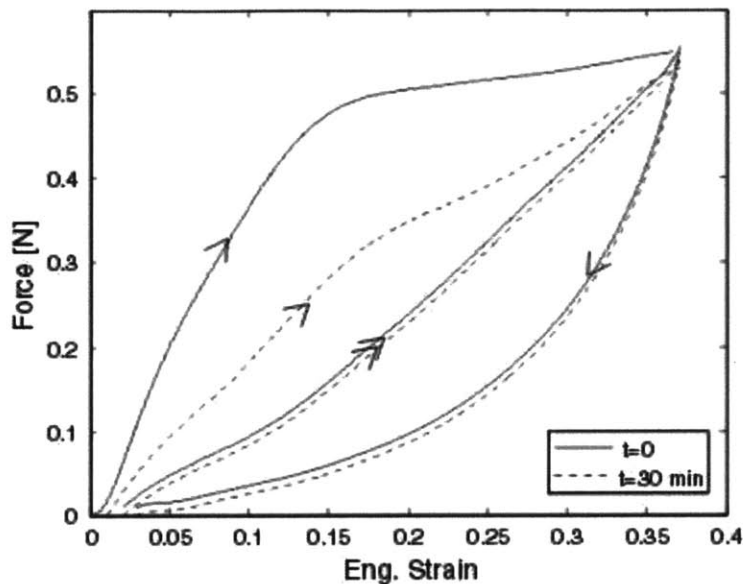
Figure 2.2: Cyclic uniaxial tension test on byssal threads of *Mytilus californianus.* The mechanical stiffness and strength are somewhat recovered after 30 minutes of relaxation between tests and the threads become more dissipative again[5, 6]. These threads contain DOPA-Fe^{3+} complexes which impart the repeatable energy dissipation observed in the tension test.

Similar metal coordination chemistries

    In a similar way that DOPA binds with trivalent metal ions, histidine is known to form

bis complex with divalent metal ions, where two His ligands complex with one metal ion.
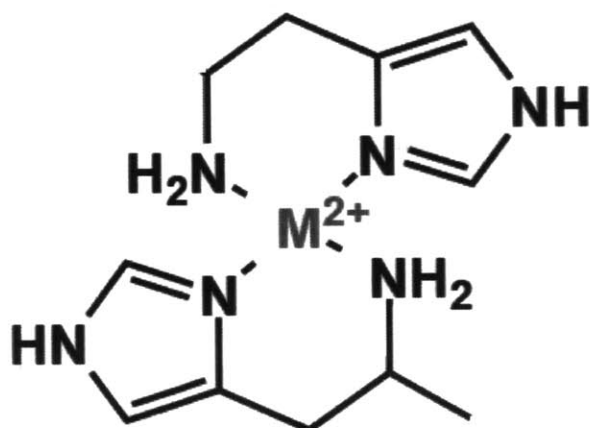
Figure 2.3: Bis complex formed from two histidine molecules and a metal ion[7].

These complexes are similar to the DOPA-metal ion complexes. They require high pH to form, form spontaneously at ambient temperature and pressures, and require substantial energy to break. They also have an intrinsic frequency which depends on the metal ion used[8]. To investigate His-metal interactions, we synthesized 4PEG decorated with histidine on the end of each arm (4PEG-His) (see Figure 2.6 a) and combined with various nickel and cobalt.
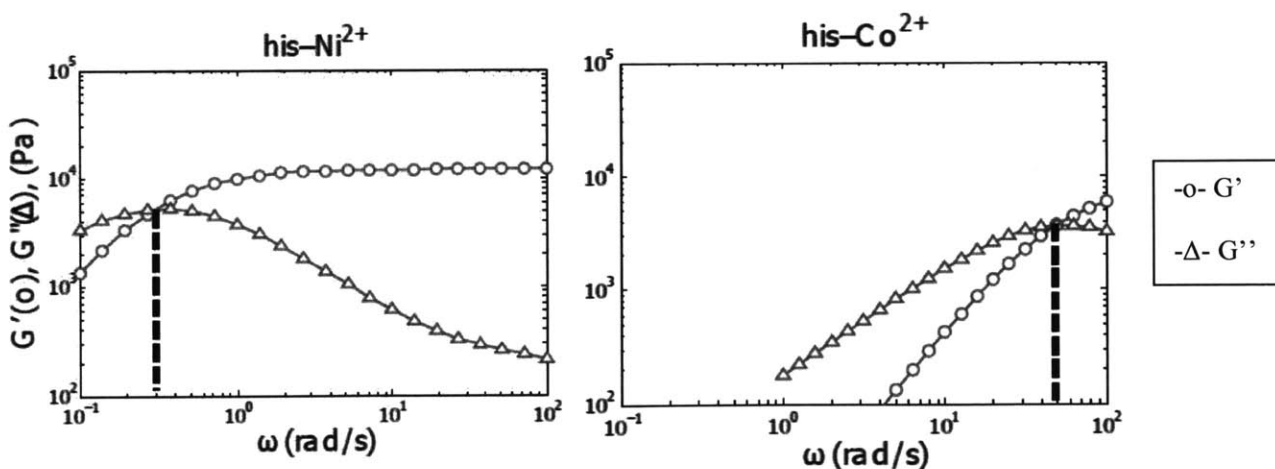
Figure 2.4: The viscoelastic response of 4PEG-His polymer melts with a) nickel and b) cobalt crosslinking metal ions. The viscoelastic response is similar but shifted in angular frequency due

to the different His-M^{2+} crosslinks. The curves collapse onto one another when they are normalized by the "relaxation time," which is the inverse of the frequency at which the viscous and elastic responses cross over one another, as indicated by the dashed lines above[9].

The mechanical response of these gels shows a peak in the loss modulus (G'') when they are evaluated using a frequency sweep. The frequency that the peak is centered around depends on the metal used. The rheometer captures the time scale over which the histidine breaks from the nickel or cobalt ions to dissipate the energy being put into the polymer gel. When the storage modulus (G') is greater than the loss modulus (G''), the polymer mix behaves as a solid, conversely, when the loss modulus is greater than the storage modulus, it behaves as a liquid.

Double network hydrogels

Doublet network (DN) hydrogels are typically composed of a neutral network within a polyelectrolyte network. This architecture yields polymer networks that are much tougher and stronger than the sum of their composite parts. The neutral polymer network is rigid and brittle and serves as sacrificial bonds under low stress. The polyelectrolyte polymer network is soft and ductile and sustains stresses over large extension[10].

The DN gels created with PEG use a slightly different motif. The neutral network of covalent bonds holds a secondary network of transient, reversible crosslinks. The neutral network is formed by using 4-arm PEG with mutually reacting functional groups: 4PEG-Amine Hydrochloride (4PEG-NH_2) and 4PEG-Succinimidyl Glutaramide (4PEG-SG) (see figure 2.5).
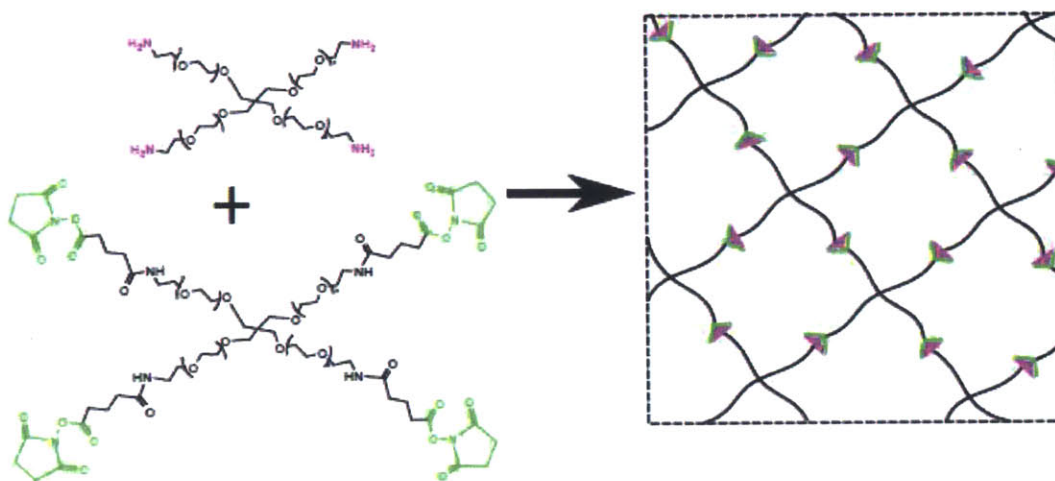
Figure 2.5: Covalent network formed by combining 4PEG-NH (purple) and 4PEG-SG polymers (green). The end groups click together by mixing the polymer with their appropriate buffers. The protecting group comes off the 4PEG-SG and an amide bond forms between arms of PEG[9].

We created double-network hydrogels by a histidine-metal 4PEG crosslinked network that serves the same role as the sacrificial bonds in the previously described DN architecture.
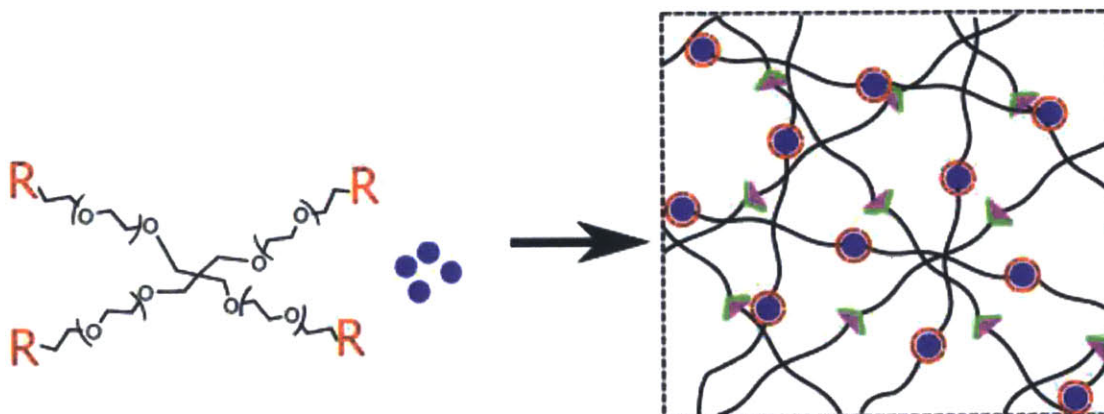
Figure 2.6: The 4PEG-His and metal ions form an interpenetrated network within the covalent network. The R groups represent histidine and the blue dots represent metal ions. The two networks combine to control the mechanical properties of the hydrogel[9].

The key difference between these DN hydrogels and those presented by Webber *et al* is that the sacrificial bonds formed in the histidine-metal ion complexes are not permanent. The energy

dissipation response to mechanical stimulation in these gels is repeatable if the gel is given a

small amount of time to reform the bonds.

3. Materials and methods

Synthesis of 4PEG-His

4PEG-NH$_2$ was purchased from JenKem Technology USA with a weight of 10kDa. It was converted to 4PEG-His through a process detailed in Donoso et al[11]. 5 grams of the 4PEG-NH2 was mixed with 1.49g Boc- His(Trt)-OH and 1.33 g BOP reagent and dissolved in 15 mL dichloromethane. 1.07 mL DIPEA was added and the reaction was allowed to proceed for 2 hours. The product was purified by precipitation in 500 mL diethyl ether once, 500 mL methanol at -20 C three times, diethyl ether once, then vacuum dried. Protecting groups were removed by a cleavage solution of 95 mL triuoroacetic acid, 2.5 mL triisopropylsilane, and 2.5 mL water for 2 hours. The solvent was removed under reduced pressure and the product purified by re-dissolving in methanol, precipitation in ether three times, and vacuum drying.

Synthesis of 4PEG-SG:4PEG-NH$_2$:4PEG-His:Ni^{2+} hydrogels

4PEG-Amine Hydrochloride (4PEG-NH$_2$) and 4PEG-Succinimidyl Glutaramide (4PEG-SG) were purchased from JenKem Technology USA at a molecular weight of 10kDa and used as received. They were dissolved into 0.1 M phosphate buffer, pH 7.4 (Sigma-Aldrich), and 0.1 M sodium acetate (Na-Ac), pH 5.2, (Sigma-Aldrich) buffer respectively to concentrations of 0.01 M. The 4PEG-His, prepared as described above, is dissolved into 0.1 M phosphate buffer to the desired concentration. Each polymer solution is mixed to equal volume, 1/3 of the unswollen volume of the hydrogel. The solutions are mixed together and poured into a mold, sealed to prevent evaporation, and cured over 4 hours at room temperature. The resulting solid gel is rinsed in MilliQ H$_2$O then soaked in solutions of NiCl$_2$.6H$_2$O diluted to the desired concentration with MilliQ H$_2$O and 0.5 M phosphate buffer for four hours each. The final hydrogels were stored under humid conditions until they could be tested.

Rheological measurements of DN hydrogels

Rheological measurements were performed using an Anton Paar, MCR 302 rheometer with parallel plate geometry. The samples were trimmed with a razor blade to the appropriate size for the testing, either 10mm or 25mm diameter. Oscillatory shear testing of the hydrogels over a range of frequencies was performed by applying a sinusoidal strain $\varepsilon = \varepsilon_o \sin(\omega t)$ where ε_o = 0.1%, to determine storage modulus (G') and loss modulus (G''). The strain of 0.1% was confirmed to be in the linear viscoelastic range through a strain sweep, which ran from 0 to 500% strain. The Anton Paar evaporation blocker system was used to reduce changes due to evaporation, further minimized by the short test duration, less than one hour. All tests were performed at 25 °C.

Compressive testing of DN hydrogels

The compressive testing was performed using a custom built Instron machine with a 10N load cell. The samples were placed between two glass cylinders and placed between the flat attachments in the machine. The samples were approximately 20 mm in diameter and 3 to 4 mm in thickness. The strain was applied at a rate of 4 mm/min to a specified strain and immediately unloaded at the same rate after reaching the prescribed strain. For most samples, two full loading cycles, one from 0 to approximately 25% strain and a second from 0 to approximately 55% strain before reaching the limits of the testing apparatus.

4. Results

Metal coordinated network within a covalent network

Combining metal ions with 4PEG-His in high pH conditions creates transient metal-histidine crosslinks. This has been shown with nickel, copper, and cobalt as the crosslinking metals. The gels created are viscous fluids with strain rate dependent viscoelastic properties, dictated by the metal ion used in the crosslinker. By mixing 4PEG-NH$_2$ and 4PEG-SG solutions with the appropriate buffers, a solution is formed that solidifies into a solid covalent network in ambient conditions. These two networks were combined into a solid material with double network architecture. The characteristic loss modulus (G'') at ~0.5 rad/s associated with nickel in the pure 4PEG-His nickel gel is present in the double network gel. In the DN gel, the storage modulus (G') has no dependence on frequency.
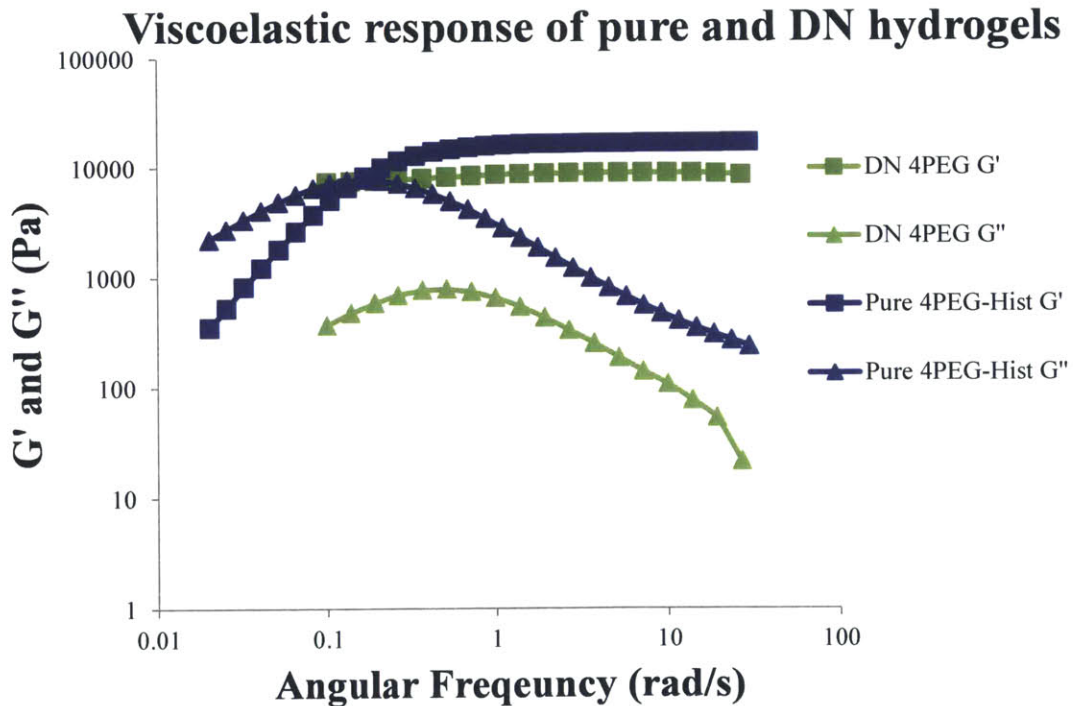
Figure 4.1: The viscous peak at low frequency that is observed in pure 4PEG-histidine gel networks is also present in double networks composed of covalently bound 4PEG and nickel

coordinated 4PEG-histidine networks. The peak is in a similar place in frequency space so the local bonding kinetics do not seem to be impaired by the presence of a covalently linked network.

Reversibility and pH sensitivity of histidine-nickel bonds

The histidine-metal bonds are known to be pH dependent. The bonds required high pH conditions to form and will not form at in low pH conditions. The pure (no covalent network) PEG-histidine nickel gels are reversible, only forming gels with the addition of strong base to the solution of PEG and nickel. When the samples are initially synthesized, they have a pH of 5.6 and the bonds are not present. We measured the initial sample before raising the pH and the peak in G'' was not present. After the gel was immersed in phosphate buffer solution, pH = 7.4, and tested again, the G'' peak was present. The gel was then placed in sodium-acetate (Na-Ac) buffer, pH = 5.4, to lower the pH for four hours and tested again; the peak was no longer present.
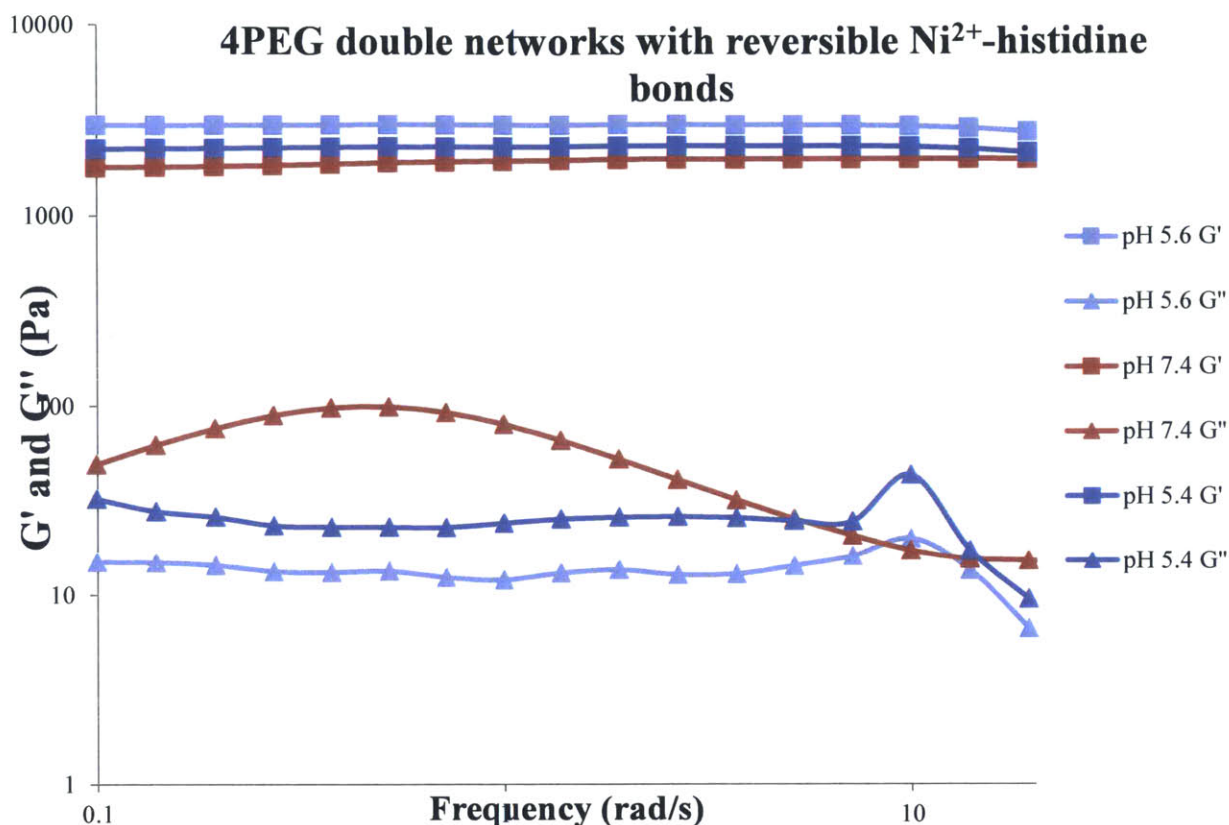
Figure 4.2: The peak in G'' is shown to be reversible by pH change, while the G' is unchanged by pH. This indicates the G'' peak is due to the histidine-Ni^{2+} bonds that are only present under basic conditions and shows the bonds exhibit previously known reversible behavior. The G' is dictated by the covalent network and does not depend on histidine-Ni^{2+} bonds. The hydrogels are initially synthesized at pH 5.6. They are soaked in phosphate buffer solution to raise it the pH to 7.4 and activate the histidine nickel bonds. Afterwards, the sample is soaked again in sodium acetate buffer to lower the pH back down to 5.4. Once the pH is lowered, the peak in G'' disappears.

Controlling the dissipation peak

Because the viscous properties are determined by the metal coordinated network, the intensity of the energy dissipation peak can be shifted by changing the amount of nickel ion present in the hydrogel. By altering the ratio of nickel to histidine end groups, the number of total histidine-nickel complexes available to break under mechanical stress changes.
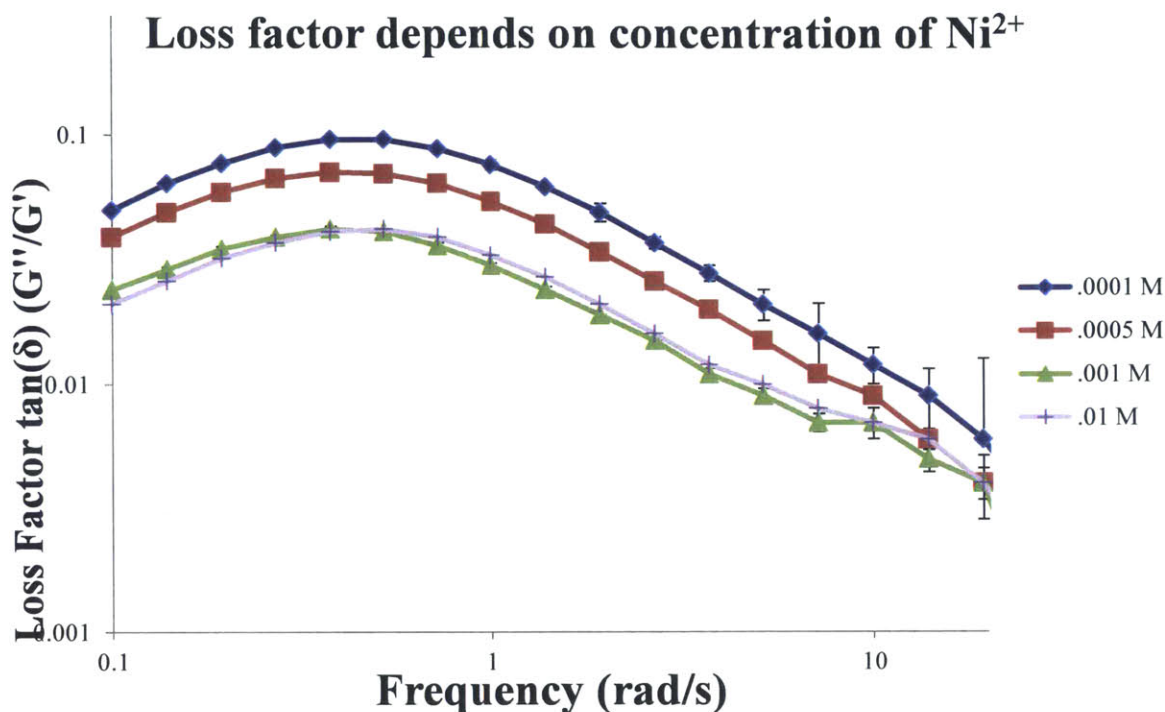
Figure 4.3: The amplitude of the loss factor ($\tan(\delta) = G''/G'$) peak varies with ratio of histidine to Ni^{2+}. The theoretical optimal ration is 2:1 histidine:Ni^{2+} because each Ni^{2+} forms bis complexes with the histidine. The increase in loss factor peak at lower concentrations indicates that not all of the histidine is binding ideally. It is possible that it is trapped due to inhomogeneities in the hydrogels or impermeabilities in the covalent network. Another explanation could be that the histidine decorated PEG chains are binding covalently with the 4PEG-SG during gel formation and consequently are not available to form complexes with the Ni^{2+}.

The most dissipative hydrogels, which have the most histidine-nickel complexes, are the gels with lower nickel concentrations. These concentrations of nickel ions represent changes in the molar ratio histidine: nickel that spans several orders of magnitude.

Ni^{2+} concentration (M)	Histidine : nickel^{2+} (1:x)
1E-4	0.019
5E-4	0.095
0.001	0.19
0.01	1.9
0.05	8.5
0.1	19.00

Table 4.1: The various concentrations of nickel converted to ratio of histidine to nickel. The experiment spanned from two nickel ions for every histidine to .02 nickel ions for each histidine. Gels formed with 0.1 M Ni^{2+} or a 1:19 ratio of histidine to nickel were visibly inhomogeneous with a different color layer at the surface and excluded from the results.

The hydrogels with the lowest ratio of histidine to nickel had the most energy dissipation capacity and the highest loss factor and loss modulus. Trials were done with concentrations between 0.01 M and 0.0001 M. The samples with concentration above 0.01 M appeared milky and were inhomogeneous with extra concentration of metal ions at the surface.
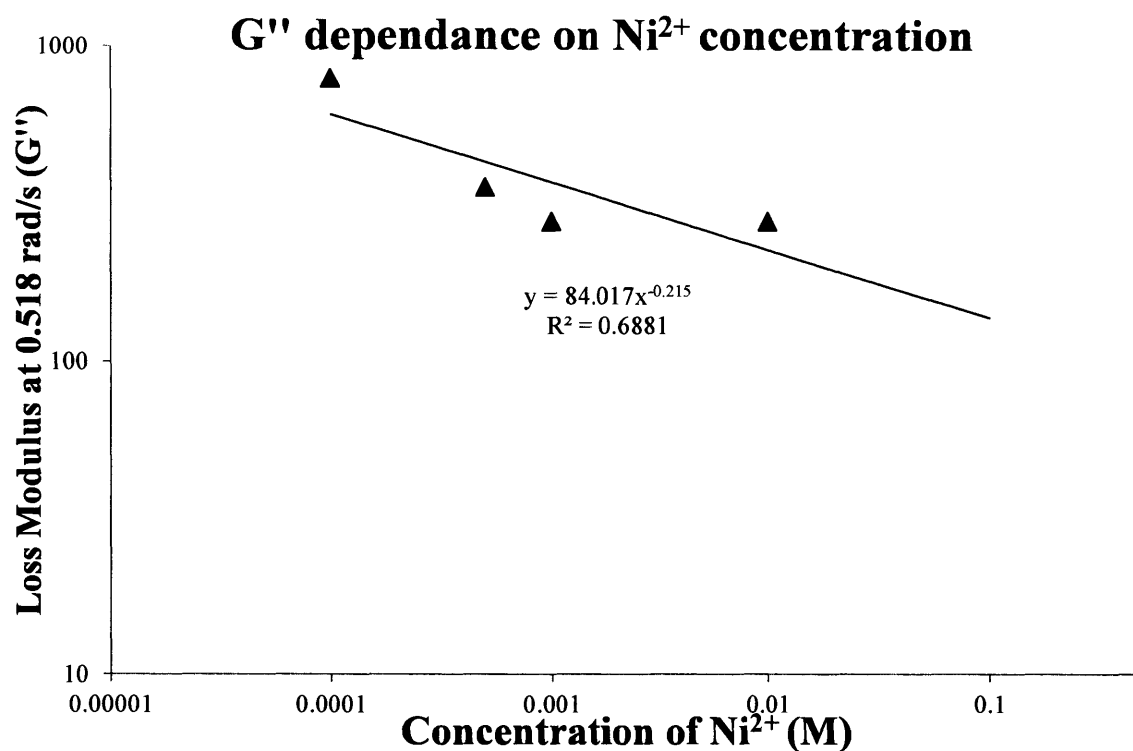
Figure 4.4: The peak G'', captured at an angular frequency of 0.518 rad/s, value scales with the concentration of Ni^{2+} according to a negative power law. It is highest when there is a low concentration of Ni^{2+} and decreases as more Ni^{2+} is added. The maximum peak is observed when the maximum amount of histidine groups are bound to the Ni^{2+} in the system and there is no excess Ni^{2+}. Excess Ni^{2+} ions form precipitate in basic solution and disrupt the mechanical response of the system.

Changing the metal ion concentration gives a degree of control over the amplitude of the loss modulus. The concentration of Ni^{2+} only affects the loss modulus. The storage modulus is unchanged by changes in Ni^{2+} concentration.

G' is independant of Ni^{2+} concentration
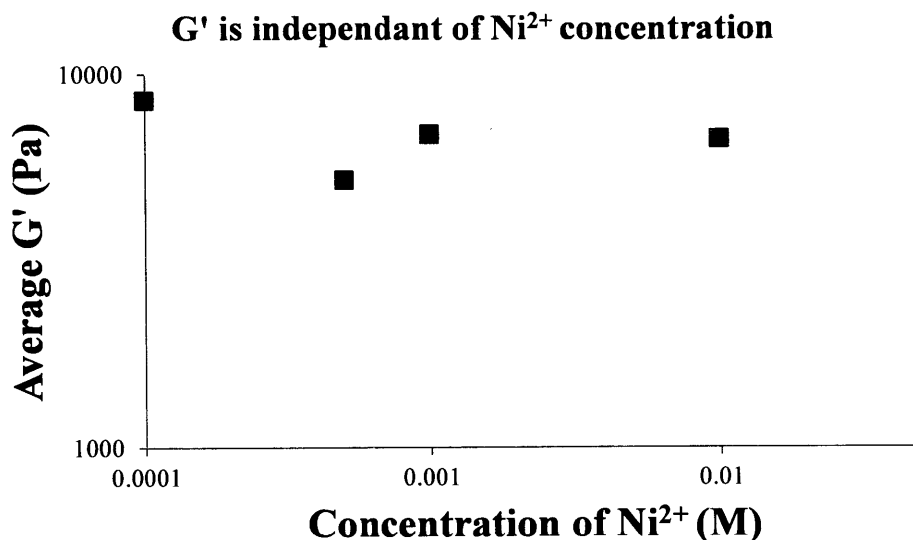
Figure 4.5: The storage modulus (G') does not vary with frequency as the loss modulus (G'') does. The storage modulus was averaged over each point on the frequency sweep and compared between hydrogels with different Ni^{2+} concentrations. G' is independent of the Ni^{2+} concentration. Note: there are error bars on all three data points but they are within the size of the icon.

Compression tests for energy dissipation

The rheological testing can determine the storage and loss moduli across a range of frequencies. It is limited at high angular frequencies because the instrument head is moving rapidly and slips off the sample. The peak in loss modulus present in 4PEG-His: Ni^{2+} bonds is at low frequencies, approximately 0.5 rad/s. In pure 4PEG-His and 4PEG-DOPA networks, changing the metal ion crosslinker changes the frequency the peak is centered around. The 4PEG-His: Cu^{2+} loss modulus peak is centered around 100 rad/s at 5 °C and it is even higher at room temperature. Because the loss modulus peak is at such high frequencies in DN 4PEG-His: Cu^{2+} hydrogels, the Anton Paar rheometer cannot resolve the signal.

Instead of relying on the rheometer to measure the energy dissipation capability of gels containing Cu^{2+}, it is possible to use cyclic compression testing. The samples were loaded and unloaded at a rate of 4 mm/minute and the stress response was recorded.

Compression response of hydrogels
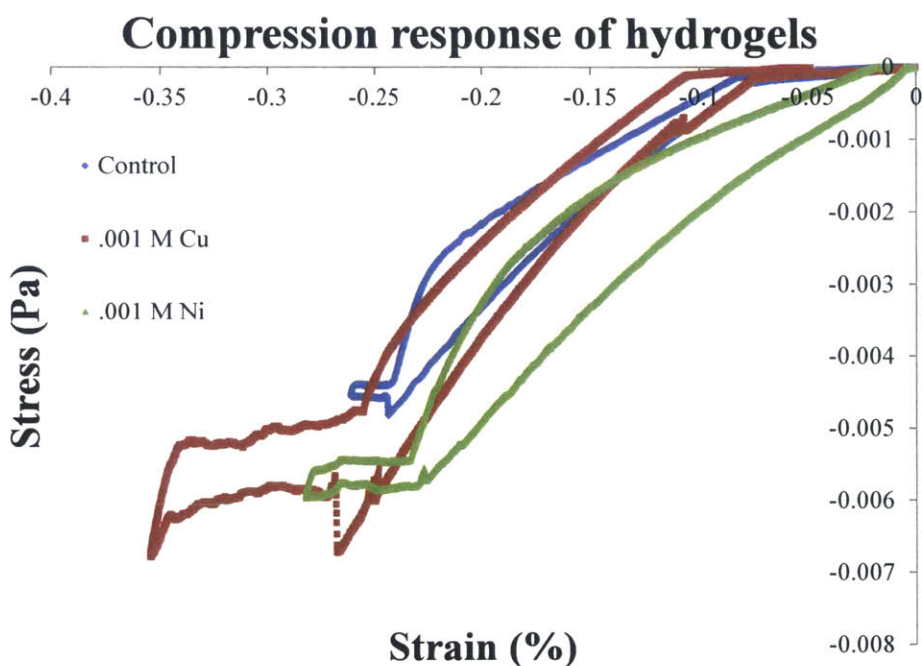
Figure 4.6: The loading and unloading of the hydrogels is negative because they were compressed and unloaded. The compressive force and compressive strain are negative and unloading is coming back towards zero. The control hydrogel has no metal ions and the other two are loaded at 0.001 M with Cu^{2+} and Ni^{2+} respectively.

The compressive loading and unloading sequence shows the stiffness of the hydrogels. The slopes of the linear region on the stress-strain curve, the stiffness, are similar for each sample. The independence of G' on metal ion used is confirmed from rheological measurements of the same samples.

To show that the Cu^{2+} gels are similarly dissipative compared to the Ni^{2+} gels, the energy dissipation can be calculated for each sample by finding the area between the loading and

unloading curves. The energy dissipation between different strains is compared for each sample. The Ni^{2+} and mixture of metal ion gels are more dissipative than the control gel, and the Cu^{2+} gel has a slope more similar to the other gels containing metal ion crosslinks than the control, although it was not tested at high strain values.
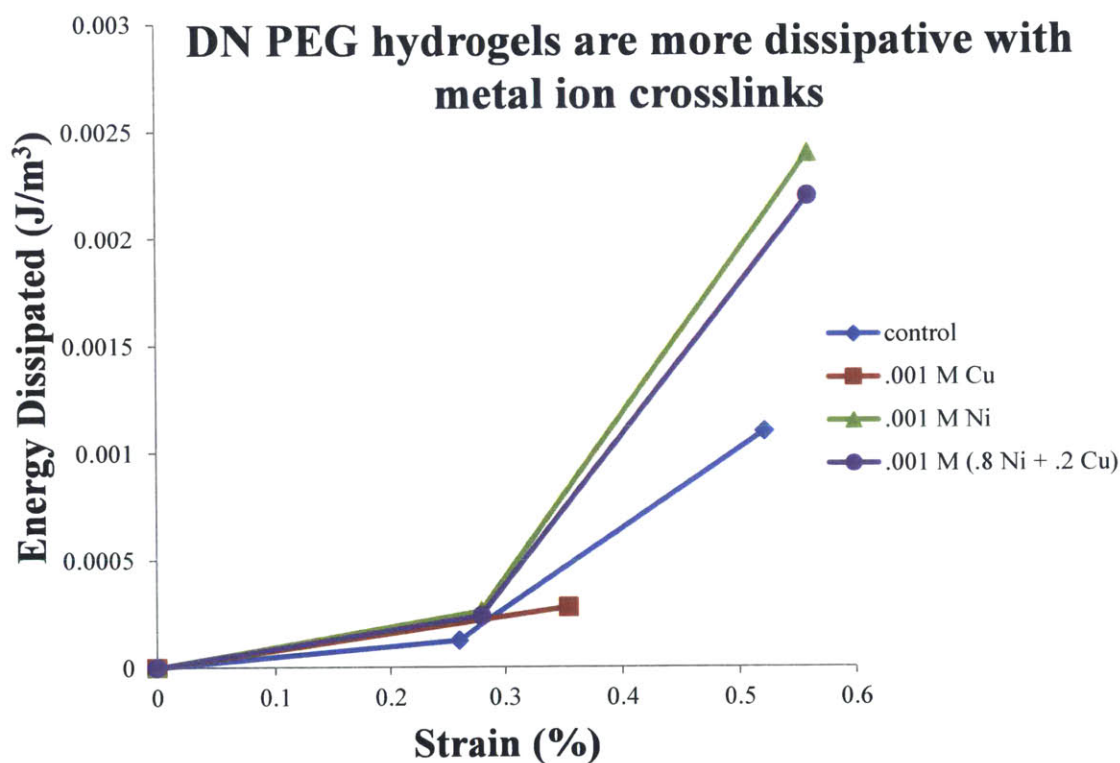

Figure 4.7: The energy dissipated due to loading and unloading at several values of strain. Three gels were tested once each at strains of 0.25 and 0.55. The purple curve is a hydrogel that has a mixture of Ni^{2+} and Cu^{2+} ions totaling a concentration of 0.001 M. The Cu^{2+} gel was only tested to a strain of 0.36 before reaching the limit of the device.

5. Discussion

The viscoelastic response of the DN hydrogels can be decomposed into the storage modulus (G') and loss modulus (G''), which can be controlled independently due to the DN architecture and different types of crosslinks present. I found that the storage modulus is independent of the presence of the transient, metal coordinated network. It remains constant if the traditional properties which dictate storage modulus response, such as degree of polymerization and crosslink density, in the covalent network are unchanged. The loss modulus depends on the metal coordinated network and relies on 4PEG-His and the metal ions in the system. The loss modulus follows the trend set by the pure 4PEG-His: M^{2+} hydrogels, where the loss modulus peak in the pure network and the double network occur at similar frequencies (see Figure 2.1). It is possible to tailor the storage and loss moduli separately by using a double-network architecture with two different types of crosslinks present in the same hydrogel.

The histidine-metal ion complexes are known to be pH sensitive. They will only form if the environment is above a certain pH threshold. The threshold is between a pH of 6 and 7, which is near the pKa of histidine. Similarly, we found the G'' behavior in these DN hydrogels to be controllable by pH. The loss modulus peak only occurs when the hydrogels have been immersed in a high pH solution, phosphate buffer solution (pH = 7.4). After they have soaked in the pH solution and the histidine-metal ion bonds have formed, the hydrogels can be removed from the solution and the bonds remain indefinitely. The samples present the loss modulus peak on a rheological frequency sweep. Before the hydrogels are soaked in the phosphate buffered solution and after they have been returned to low pH via sodium-acetate buffer (pH = 5.2), the loss modulus is constant and below the high pH peak when probed via frequency sweep. This

result helps attribute the peak in loss modulus to the histidine-nickel bonds between 4PEG molecules.

The loss modulus is not constant in frequency space, and peaks at a certain frequency. Researchers believe the frequency of the peak is dictated by the kinetics of the bond between the histidine ligand and the metal ion. By changing the metal ion, the kinetics change and therefore the resonant frequency of the bonds change. The metal ion concentration dictates the intensity of the loss modulus peak. Surprisingly, the ratio between histidine and nickel required to maximize the loss modulus is approximately 1:0.02 or 50:1. In the ideal case, the optimal ratio would be 2:1, because each nickel ion forms a complex with two histidine molecules. However, much more histidine is required to optimally bind with the nickel present in the hydrogel. This could be because some PEG chains with histidine end groups are trapped in the DN architecture and are kinetically unavailable to take part in forming a bis complex between another histidine end group and a nickel ion. It is also possible that in the synthesis process some of the histidine end groups are modified by the phosphate buffer solution, form covalent bonds with the succinimidyl glutaramide end groups, and contribute to the covalent network instead of the transient one. This would further increase the likelihood of kinetic traps preventing all free histidine to bind with nickel ions inside the gel.

We found that the storage modulus is unaffected by the changes in nickel concentration as long as the concentration is below a specific amount. As the concentration gets above 0.01 M, the gels become visibly inhomogeneous. After being soaked in basic solution to form the histidine-metal bonds, there is an outer shell of more intense color (green for gels containing nickel, blue for gels containing copper) and an inner core of less intense color. At the surface, the excess metal ions form precipitate with the basic solution. We hypothesize that these metal

precipitates weaken the mechanical integrity of the hydrogels, but this phenomena was not tested extensively.

The loss modulus was found to decrease as the concentration of Ni^{2+} ions increased past 0.0001 M according to a power law. In the case of nickel ions, the exponent was experimentally determined to be -0.2. Further study is needed to understand what determines the scaling and what mechanism causes the decrease. The metal ion could be changed to see if the exponential depends on the metal ion or if the phenomenon is consistent within the 4PEG-His double network architecture. A possibility is the unbound ions are forming precipitate and disrupting the mechanical properties of the hydrogel, including energy dissipation capability of the histidine-nickel complexes.

It was difficult to resolve the histidine-Cu^{2+} loss modulus peak within the double network hydrogel. It is only present at high frequencies, even at low temperatures, and the speed required from the rheometer at those high frequencies caused slippage at the interface between the tool and the sample, rendering the data unusable. Through the use of cyclic loading tests on the Instron, hydrogels with Ni^{2+}, Cu^{2+}, and a mixture of Ni^{2+} and Cu^{2+} crosslinks were shown to have similar energy dissipation per volume. This energy dissipation was greater than that of the control gel with no metal ions in it, especially at high strain rates. The hydrogels relying on Cu^{2+} as the metal ion crosslinker behave similarly to the ones using Ni^{2+}, in that they have specific energy dissipation properties. Despite that it is more difficult to see the energy dissipation at a particular frequency with simple uniaxial compression compared to rheometry; the macroscopic mechanical properties are still present in 4PEG-His double network architecture with metals other than nickel as the crosslinking metal ion.

In the pure 4PEG-His: M^{2+} networks, it is possible to combine different divalent metal ion crosslinkers in the same sample. For example, if the polymer network contains 75% Ni^{2+} and 25% Co^{2+}, it exhibits twin peaks in the loss modulus, as seen in Figure 5.1.
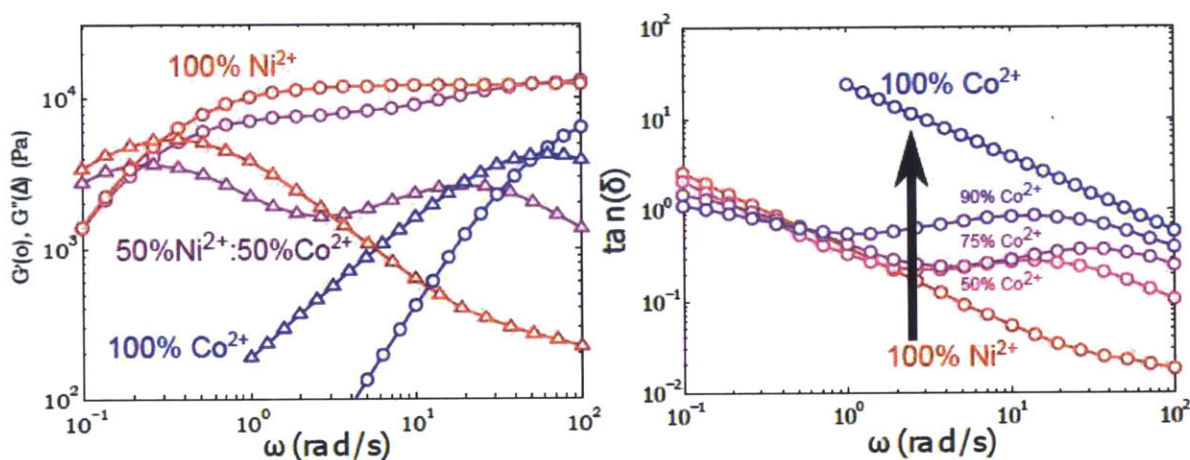

Figure 5.1: a) Two local maxima in G'', each corresponding to the specific relaxation time of the pure 4PEG-Hist:M^{2+} network, in a 50-50 mixture of Ni^{2+} and Co^{2+}. b) Changing the ratio of Ni^{2+} to Co^{2+} directly controls the loss modulus peaks[12].

The double network hydrogels follow the loss modulus behavior of the pure network for single metal ion crosslinks. It follows that a doublet network with two different metal ion crosslinks would also exhibit twin peaks in the loss modulus. The DN hydrogels with a mixture of nickel and copper ions showed similar energy dissipation to DN gels with only nickel or copper on the cyclic compression tests. To accurately measure the changes in loss modulus in the frequency domain, there are a few techniques that could be employed. Increasing the diameter of the samples to fit under the 50mm diameter testing head may help reduce the noise in the measurements. Increasing the normal force applied by the measuring head of the rheometer may also help reduce slippage between the head and the sample.

Mechanical testing was done using rheometry and compressive testing. Tensile testing is also desirable but it is difficult with the instruments available. The DN hydrogels were cast into dogbone shapes for tensile testing but could not be mounted in the Instron reliably. They either slipped out of the grips or were crushed when tightened into grips. Over tightening resulted in stress concentrations and sample failure in the thick region of the dogbone instead of the bulk of the sample.

Another tool that may help link the changes in mechanical response to the changes in molecular organization is ultraviolet and visible light (UV-vis) spectroscopy. Metal ion solutions have a distinct absorption spectrum and when the metal ions are bound to histidine the absorption spectra change. There is a difference between DN hydrogels soaked with a 0.001 M solution of Cu^{2+} at high and low pH that is visible to the naked eye. The difference between high and low pH gels with 0.001 M Ni^{2+} is less noticeable because the absorption is less intense in both the pure solution and the histidine-nickel complex. The peak shift may be quantifiable using UV-vis spectroscopy. The changes in mechanical response are already correlated with basic conditions which are believed to induce formation of the histidine-metal complexes. Correlating this mechanical change with changes in the visible color characteristics of the gel would be a powerful tool. It may be possible to measure the color spectrum of a gel in real time and see the local energy dissipation as the bonds break and reform. One could easily see if the gels energy dissipation capacity had been spent and they needed time in an unloaded environment to reform the bonds and recover the energy dissipation capability.

If the mechanical absorption spectrum can be optimized and easily controlled, it could be possible to tailor these double network hydrogels for use as acoustic dampening materials. The

frequencies that they attenuate would be highly tunable and specific; allowing sounds at all other

frequencies through unaltered.

References

1. Lee, B. P., Messersmith, P. B., Israelachvili, J. N., & Waite, J. H. (2011). Mussel-inspired adhesives and coatings. *Annual review of materials research, 41*, 99.
2. Holten-Andersen, N., Jaishankar, A., Harrington, M. J., Fullenkamp, D. E., DiMarco, G., He, L., ... & Lee, K. Y. C. (2014). Metal-coordination: using one of nature's tricks to control soft material mechanics. *Journal of Materials Chemistry B, 2*(17), 2467-2472.
3. Webber, R. E., Creton, C., Brown, H. R., & Gong, J. P. (2007). Large strain hysteresis and mullins effect of tough double-network hydrogels. *Macromolecules, 40*(8), 2919-2927.
4. Zeng, H., Hwang, D. S., Israelachvili, J. N., & Waite, J. H. (2010). Strong reversible Fe3+-mediated bridging between dopa-containing protein films in water. *Proceedings of the National Academy of Sciences, 107*(29), 12850-12853.
5. Bertoldi, K., & Boyce, M. C. (2007). Mechanics of the hysteretic large strain behavior of mussel byssus threads. *Journal of Materials Science, 42*(21), 8943-8956.
6. Moeser, G. M., Leba, H., & Carrington, E. (2006). Seasonal influence of wave action on thread production in Mytilus edulis. *Journal of Experimental Biology, 209*(5), 881-890.
7. Miura, T., Satoh, T., Hori-i, A., & Takeuchi, H. (1998). Raman marker bands of metal coordination sites of histidine side chains in peptides and proteins. *Journal of Raman spectroscopy, 29*(1), 41-47.
8. Fullenkamp, D. E., He, L., Barrett, D. G., Burghardt, W. R., & Messersmith, P. B. (2013). Mussel-inspired histidine-based transient network metal coordination hydrogels. *Macromolecules, 46*(3), 1167-1174
9. Grindy, S. C., Learsch, R., Mozhdehi, D., Cheng J., Barrett, D. G., Guan, Z., Messersmith, P. B., & Holten-Andersen, N. Disentangling structure from function: controlling hierarchal polymer mechanics with bio-inspired metal-coordination dynamics. *In preparation.*
10. Gong, J. P. (2010). Why are double network hydrogels so tough?. *Soft Matter, 6*(12), 2583-2590.
11. Donoso, R., Cárdenas, C., & Fuentealba, P. (2014). Article Previous Article Next Article Table of Contents Ab Initio Molecular Dynamics Study of Small Alkali Metal Clusters.
12. Grindy, S., Learsch R. et al, *Gordon Research Conference on Bioinspired Materials.* (2014).

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