Spring 2023

Anti-Freeze Double Network Hydrogels

William Gross
wg16@uakron.edu

Anti-Freeze Double Network Hydrogels

University of Akron Williams Honors College

William Gross IV

4/21/2023

Abstract

Three polyethylene glycol (PEG) based crosslinkers with repeat unit nominal weights of 62.1, 200, and 400 g/mol in agar/PAM double network (DN) hydrogels were used to study the effect of different crosslinkers on thermal and mechanical properties. Using the one-pot synthesis method, hydrogels with the PEG crosslinkers or methylene-bis-acrylamide (MBAA) were made via sol-gel transition for formation of agar network, and photopolymerization of the PAM network using Irgacure 2959 as photo initiator. Tensile and compression tests were performed on as prepared, swollen, and swollen & cooled specimens of each hydrogel. Freezing time and thermal imaging were performed in -20 °C freezer, and thawing at room temperature followed freezing in -60 °C freezer. MBAA then PEG based crosslinkers with increasing molecular weight increased freezing time (normalized 1 hr, 1.9 hr, 2.9 hr, 3.8 hr) and decreased heat conductivity which may result from London dispersion forces, crosslinker hydrogen bonds with water, and porosity. Under tension and compression, PEG_{400}-INA and EGINA based crosslinker experienced better trends from colder temperatures on mechanical properties than MBAA. They also experienced decreased swelling ratios of 6.11, 5.65, 5.20, 5.75 from MBAA to PEG_{400}-INA when compared to that of PAM SN 11.69. This is work will inspire researchers to optimize PEG based crosslinkers in as prepared hydrogels, understand their anti-freezing mechanisms, and find applications in low temperature environments such as supercapacitors, and deep ocean.

Keywords: double network hydrogel, crosslink, anti-freeze, swelling

Executive Summary

Hydrogels are three-dimensional cross-linked networks that hold a large amount of water (50–90%). Their extensive applications in tissue engineering, drug delivery systems, electronics, and many other fields have made them widely studied and modified to exhibit favorable properties. One such unfavorable property of hydrogels is their freezing point being near water, 0 °C, due to their high quantity of water. The addition of salts to hydrogels has been researched and showed to be a promising avenue for lowering the freezing temperature to as low as -60 °C. This research examines a new method, studying the effect of the polymer networks in a double network (DN) Agar/PAM hydrogel on anti-freezing behavior. The introduction of three PEG based crosslinkers with increasing molecular weights: EGINA, PEG_{200}-INA, and PEG_{400}-INA, are substituted for the standard MBAA crosslinker used in crosslinking PAM.

The effect the different crosslinkers have on thermal properties were studied from measuring freezing times of circular specimens 1mm thick and 10 mm in diameter in a -20 °C freezer. Over three trials, the freezing times were normalized for MBAA exhibiting a 1 hour freezing time, and the PEG based crosslinkers with increasing molecular weight exhibiting freezing times of 1.9 hours, 2.9 hours, and 3.8 hours. To understand the freezing process of the hydrogels, thermal imaging was taken of samples in a -20 °C for 8 minutes using a FLIR thermal imaging camera. Thermal imaging showed that the MBAA crosslinked hydrogel was quicker to equilibrate to the lower temperature in the freezer. Thermal imaging was also taken of specimens thawing for 3 minutes at room temperature after being placed in a -60 °C freezer for 1 minute, and all samples were fully frozen. While thawing the PEG crosslinked samples with increasing molecular weight equilibrated to room temperature slower than MBAA. These results showed that PEG crosslinked hydrogels with increasing molecular weight may not only have lower freezing points, but also decreased heat conductivity than MBAA crosslinked hydrogels. The hydrophilic nature of PEG disrupts water-water intermolecular bonds, and three anti-freezing mechanisms are proposed to explain the trends in freezing and heat conductivity. One mechanism is the increased molecular weight of crosslinkers from MBAA to PEG_{400}-INA (154.2, 348.34, 486.24, and 686.24 g/mol) increases surface area and London dispersion forces for interaction between crosslinks and water, decreasing water-water intermolecular interactions. A second mechanism is the addition of oxygen molecules in the crosslinks increase hydrogen bonding between water and crosslinks. A third mechanism that is least dominant but still feasible, is the increased porosity of the hydrogels via increased crosslinking degree decreases water-water interactions between pores. Research should be performed to find freezing points of the hydrogels to confirm the increased freezing time was not due to reduced heat conductivity. Also, DSC tests should be performed to find the heat conductivity of the hydrogels.

Mechanical properties of MBAA and PEG based crosslinkers were observed at different conditions. Firstly, the effect of UV light exposure (365 nm wavelength, 8W) was examined on MBAA crosslinked hydrogels. Three samples with identical fabrication procedure, except each was exposed to wither one, two, and four hours of UV light which affected the synthesis of the crosslinked PAM network in a cylindrical mold 10 mm in diameter. One hour of UV exposure was very weak and fractured at only 25% compressive strain and 0.055 MPa stress. The

weakness of the hydrogel is attributed to the low crosslinking density of the PAM network. Two hours of exposure proved optimal as it reached 95% compressive strain (maximum achievable for safety of machine) and 7.87 MPa stress. After 4 hours of UV light exposure the hydrogel weakened, it reached 95% compressive strain but only achieved 6.89 MPa stress. This demonstrates that there is an inflection point of mechanical strength with increasing crosslinked degree.

The effect of temperature and swelling on mechanical strength was studied with the different crosslinked hydrogels. First, a tension test was performed on fabricated samples with different crosslinkers at room temperature, and after being in -20 °C freezer for 10 minutes. MBAA displayed the best results with stresses & strains of 0.656MPa & 22.02 mm/mm, 0.689MPa & 25.6 mm/mm at room temperature and cooled respectively. At room temperature EGINA and PEG_{400}-INA displayed 0.247 MPa, 5.03 mm/mm and 0.246 MPa, 4.74 mm/mm respectively. After being cooled PEG_{400}-INA had the 2nd best stress-strain with EGINA close behind (0.36 MPa, 5.06 mm/mm and 0.352 MPa, 6.09 mm/mm respectively). PEG_{200}-INA was the weakest by far, only having 0.224 MPa, 1.97 mm/mm and 0.22 MPa, 2.03 mm/mm at room temperature and cooled. Although MBAA had the best mechanical properties at room temperature and cooled, EGINA and PEG_{400}-INA specimens experienced the greatest increase in maximum strain (16% increase compared to 21% and 18% respectively). PEG_{400}-INA and EGINA specimens also experienced a greater increase in stress at colder temperature (46% and 42% increases compared to only 5% increase respectively). These results may be due to increased brittleness of the agar and/or PAM network in EGINA and PEG_{400}-INA. This is most apparent in the increase of Modulus in EGINA and PEG_{400}-INA at colder temperature, and PEG_{400}-INA exhibiting more apparent yielding behavior when cooled.

Second, a compression test was performed on fabricated, swollen, and swollen & cooled hydrogel samples with different crosslinkers. Under compression, fabricated PEG_{400}-INA was the strongest (95% strain, 6.69 MPa) followed by MBAA (95% strain, 6.638MPa), EGINA (95% strain, 6.634 MPa), and PEG_{200}-INA (61% strain, 0.508 MPa). When swollen, PEG_{400}-INA experienced the least decrease in max stress (10%) followed by EGINA (17%), PEG_{200}-INA (35%), and MBAA (46%). Swollen & cooled, again PEG_{400}-INA experienced the littlest decrease in max stress compared to swollen (24%) followed by MBAA (54%), and EGINA (65%). PEG_{200}-INA increased in max stress when swollen & cooled compared to swollen (12% increase), however, its stress and strain were very poor compared to its counterparts. When compared to fabricated, PEG_{400}-INA swollen & cooled easily outperformed the other hydrogels with only a 32% decrease in max stress followed by EGINA (71%) and MBAA (75%). The increase in strength under compression may be due to PEG based crosslinkers creating a more brittle PAM network compared to MBAA. When cooled, performance of PEG_{400}-INA is most likely least effected due to its high heat resistance and mitigation of ice crystal formation that could cause deformations in the hydrogel networks, resulting in a weaker specimen.

The swelling of the different crosslinked hydrogels was briefly examined with the MBAA, EGINA, PEG_{200}-INA, and PEG_{400}-INA crosslinkers exhibiting swelling ratios of 6.11, 5.65, 5.20, and 5.83 after being dried in a 70 °C oven for 23 hours, and allowed to swell in DI water

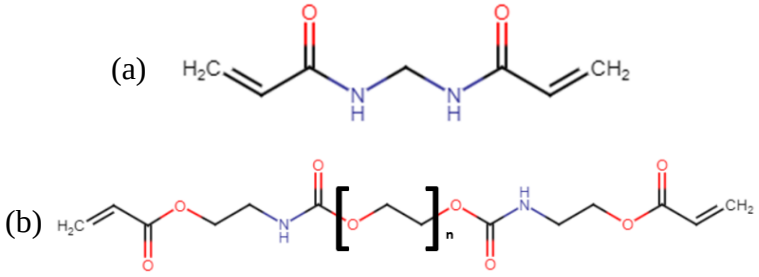
for 45.5 hours. This is compared to PAM SN hydrogel which has a swelling ratio of 11.69. Decreased swelling ability is favorable in most applications, and mostly a result of increased crosslinking. However, these tests did not show any trend between swelling, crosslinking, and mechanical testing (especially PEG_{200}-INA). This should be further researched through techniques such as H-NMR, SEM, and porosity measurements to research the structure of the PAM network with different crosslinkers.

The degree of crosslinking has an inflection point where mechanical strength and toughness decreases with increased mol% crosslinker of monomer (MBAA or PEG's mol% of acrylamide monomer). Agar/PAM crosslinked with MBAA hydrogel fabrication had been optimized for performance prior to this research. The fabrication of PEG based crosslinkers was not optimized and therefore could still increase its fabricated mechanical properties. Despite this, PEG_{400}-INA and EGINA crosslinkers have displayed promise for future research of anti-freezing double network hydrogels.

Introduction

Hydrogels are three-dimensional cross-linked networks that hold a large amount of water (50–90%). [6] Their soft and flexible physical properties along with their chemical properties make them favorable in many modern engineering applications. Some of which are contact lenses, drug delivery systems, absorbents, and tissue engineering. The goal of some current research in hydrogels is to have them exhibit stronger physical properties such as strength, self-healing, and toughness. Also, due to their makeup of mostly water, they tend to freeze in subzero temperatures which weakens them. This research will look into the effect of different crosslinkers on hydrogels strength, toughness, and anti-freezing properties.

Firstly, the effect of crosslinking time on hydrogel strength and toughness will briefly be explored. This was performed by the synthesis of an Agar/Polyacrylamide (Agar/PAM) double network (DN) hydrogel crosslinked with methylene-bis-acrylamide (MBAA) via the one-pot method[6], and observing the compression performance at three different UV exposure times which affects the crosslinking density of the PAM network.



Figures 1a and b. Chemical structure of MBAA (a) and PEG (b) based crosslinkers. For EGINA, PEG_{200}-INA, and PEG_{400}-INA, crosslinkers number of repeating units (n) average is 1, 4.54, and 9.08 respectively.

Second, the substitution of three different polyethylene glycol (PEG) based crosslinkers, EGINA, PEG_{200}-INA and PEG_{400}-INA (Figure 1b) for MBAA (Figure 1a), will study the effect of different crosslinkers on freezing mitigation, swelling, strength, and toughness of Agar/PAM DN hydrogels. In this text the use of EGINA, PEG_{200}-INA, PEG_{400}-INA, and MBAA will denote the use of Agar/PAM DN hydrogels with these crosslinkers. MBAA has been widely researched as a crosslinker for acrylamide networks, and optimized by Chen et al. [6] for Agar/PAN DN, however, optimization for wt% of PEG based crosslinker was not performed for this work. However, the good performance of the PEG based crosslinkers despite this is promising, especially since the wt% of crosslinkers to acrylamide (AM) has been shown to have a direct correlation to hydrogel mechanical properties. [1]

Most research of freezing point depression in hydrogels has been through the addition of salts to water within the hydrogel system. This has had promising effects, with hydrogels having freezing points as low as -57 °C by the addition of $CaCl_2$. [8] However, the use of salts in hydrogels is not favorable for some applications, especially biologically, as salts may leech from the hydrogel into surrounding tissue which may affect the patient and decrease the thermal properties of the hydrogel. In electrical applications salts may be favorable for their improved

conductance [9], however, for applications requiring high resistance there must be another way to lower the freezing point. This work researched the effect of different crosslinkers on the thermal and mechanical properties of an Agar/PAM DN hydrogel. The mechanical properties of MBAA as a crosslinker for PAM has been widely studied, in this work 3 PEG derivative crosslinkers with varying repeat EGINA units are introduced. Their effect on thermal properties are studied by thermal imaging in the freezing and thawing process, and timing a 1mm thick sample freezing. The mechanical properties under tension are studied from fabricated samples at room temperature and after being cooled. Mechanical properties under compression are studied at fabrication, swollen, and swollen & cooled. Swelling is an important factor in hydrogel mechanical strength and is directly related to the crosslinking degree of hydrogels, it also affects the thermal properties of hydrogels.

Experimental Procedure

I. One-Pot Synthesis of Double Network Agar/PAM MBAA Hydrogel

All chemicals were supplied by Sigma Aldrich. Weighed 100 mg agar (gel strength >300 g/cm^2, melting point 85-95 °C) 900 mg AM, 0.0284 g 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959, 1 mol% AM) into 20 mL vial. Weighed 10 mg of N-N'-methylene-bis-acrylamide (MBAA) into another 20 mL vial and added by pipette 1 mL of DI H_2O (10 mg mL^{-1} solution). Sonicated solution for 10–30 seconds and solute fully dissolved in water. Pipetted 59 μL of solution (0.03 mol% AM) into 20 mL vial containing agar and other constituents. Pipetted 5 mL of DI H_2O into precursor vial containing agar and other constituents, then sonicated for 15-30 seconds. If left to sit, precursor formed a cloudy viscous substance at bottom and water on top which was caused by agar. Added magnetic stir bar to precursor vial and sealed with cap. Placed sealed vial in hot water bath preheated to 90 °C (water level in bath with vial inserted was less than cap height) with magnetic stirring on for 5 minutes and solution was transparent. Preheated molds and 6 mL syringe with needle for solution transfer in 70 °C oven for at least 5 minutes (refer below for cylindrical and sheet mold preparation). After precursor heated for 5 minutes, removed molds and syringe from oven, and solution from hot water bath. Quickly took cap off precursor and used syringe to draw up solution. Transferred 2.5-3 mL of solution into each mold (one precursor was enough for 2 molds). After transfer, cleaned syringe of solution by drawing up and dispelling hot water from hot water bath twice (this allowed reuse of syringe). Closed opening of molds with cling wrap and allowed to sit for 5 minutes at room temperature (opening face up) resulting in a transparent near solid solution which signified beginning of formation of agar network by sol-gel transition. Placed molds under 365 nm wavelength, 8 W UV lamp (molds placed on side with greatest surface area showing) and covered with cardboard box for personal protection. UV exposure 1.5 hours for sheet molds and 2 hours for cylindrical molds (or different time if noted), and flipped molds to other side every 30 minutes. After 1.5 hours sheet molds formed a thin line of clear solid closest to opening while bulk of solid was opaque (due to oxygen disrupting radical polymerization used in crosslinking PAM chain). Stored sheet mold on one side of plastic, wrapped in cling wrap, sealed in plastic bag, and placed in 2 °C fridge to reduce water loss in hydrogel before running

experiments. Cylindrical molds were kept in syringe with opening covered by plastic wrap and sealed in a plastic bag in 2 °C fridge.

II. Double Network Agar/PAM PEG Hydrogel One-Pot Synthesis

Synthesis of PEG crosslinked DN hydrogel followed same one-pot method described above except addition of MBAA solution to precursor. EGINA, PEG_{200}-INA, and PEG_{400}-INA were synthesized by Dr. Dong Zhang and stored in -20 °C freezer. For EGINA and PEG_{200}-INA crosslinked hydrogels, allowed to sit in room temperature for one minute to reduce viscosity and, pipetted 59 μL of crosslinker into precursor. Due to high viscosity of PEG_{400}-INA crosslinker, scooped estimated 59 μL amount of crosslinker into/onto pipette tip and swirled into precursor (water added to precursor before crosslinker to easily remove PEG_{400}-INA from pipette tip).

III. Sheet Mold Preparation

Prepared sheet mold using 2 pieces of glass and transparent non-stick plastic each with dimensions of 7 cm x 8.5 cm. Teflon separator had 0.5cm opening cut in top, and outer dimensions of 7 cm x 8.5 cm with 0.7 cm thick border and middle cut out. Mold assembly followed Glass|Plastic|Separator|Plastic|Glass. Mold held together using 6 binder clips (2 on every side excluding side with opening). Transferred hot precursor into mold by inserting needle into opening in Teflon separator and filled with 2.5-3 ml of solution.

IV. Cylinder Mold Preparation

Cylinder mold was formed using a 3 mL drawn syringe (10 mm ID). After 2.5-3 mL of solution was inserted into syringe, closed syringe to point where solution just entered the tip to decrease contact of hydrogel with air.

V. Qualitative Anti-Freezing Time

Retrieved pre-synthesized sheet mold (1 mm thick) hydrogel samples: Agar/PAM with MBAA 1, Agar/PAM with MBAA 2, Agar/PAM with EGINA, Agar/PAM with PEG_{200}-INA, and Agar/PAM with PEG_{400}-INA from fridge (2 °C). Cut two circles, 10 mm in diameter from each sample and placed onto glass staging area organized by column with labeling above the samples. Took cut outs from different areas on sample (i.e. one lower and one higher from mold). Placed glass stage with samples into -20 °C freezer on top of plastic container (don't want glass directly in contact with freezer trays or any high heat conductive material) and began timing when freezer closed. Observed samples at 2 minutes with no difference in samples recorded (all are colorless and transparent). Observed samples at 6 minutes, noticed white crystals form in/on Agar/PAM 1 and Agar/PAM 2 samples but no change in other samples. Quickly took glass staging out of freezer using a pre-cooled Kimwipe in the freezer (avoid contact with warm hands) and placed onto colored paper. Took picture of samples for records. Returned samples to freezer. At 15 minutes observed white crystals formed in/on Agar/PAM 1, Agar/PAM 2, & EGINA samples but no observable difference in PEG_{200}-INA and PEG_{400}-INA samples. Investigated following same procedure from 6 minutes and placed back into freezer. At 24 minutes observed white crystals formed in/on all samples. Prepared two tweezers by rubbing ends of them on ice in freezer to cool them down. Used tweezers to pick up and try bending all

samples. Agar/PAM 1 and Agar/PAM 2 samples were stuck to glass sheet and fully hardened. EGINA, PEG_{200}-INA, and PEG_{400}-INA samples were able to be picked up from glass sheet and were still flexible. Could scrape crystals off top of Agar/PAM with EGINA, PEG_{200}-INA, and PEG_{400}-INA samples revealing colorless transparent hydrogel.

VI. Anti-Freezing Thermal Imaging

One sample each of Agar/PAM with 4 different crosslinkers (MBAA, EGINA, PEG_{200}-INA, PEG_{400}-INA) placed on glass sheet. Placed in cardboard box with top cut out holding thermal camera in place facing samples. Placed samples inside cardboard box with thermal camera into -20 °C freezer and began recorded for 10 minutes.

Using same setup, recorded samples in -60 °C freezer for 1 minute. Then removed samples and recorded at room temperature for 2 minutes 42 seconds.

VII. Tension Test

Used Instron 3345 with BlueHill 3 software. Software calculated Modulus (if applicable) from 10%-40% of max strain and area under stress-strain curve. Software also recorded force applied, stress, strain, stress at yield (if applicable). Sample cut from sheet mold (1 mm thick), refer to Figure 2 for dimensions.

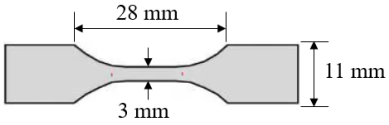


Figure 2. Dimensions of tension test specimens.

Strain rate set at 400 mm/min for all tests. For room temperature samples with different crosslinkers, sample placed at room temperature for at least 5 minutes once removed from fridge before being placed in machine to run test. Specimen cut from sample using cutter with specimen dimensions. Before test, grips cleaned with Kimwipe and machine calibrated. Once calibrated and cleaned, placed room temperature sample in grips and starting hydraulic arm height set where testing specimen was straight upon eye inspection. Room temperature specimens stick to grips, applied small amount of water to grip sections of hydrogel to easily insert specimen into grips (reduced risk of pre-straining specimen). Zeroed strain and force in BlueHill 3 software. Started software at 400 mm/min strain rate and stopped after sample fractured.

Ran same procedure on all samples with 4 different crosslinkers at lower temperature. Wrapped cut specimen in cling wrap and placed in -20 °C freezer for 10 minutes. Cooled down a pair of tweezers in freezer as well. After 10 minutes, removed specimen from freezer and quickly transferred using cooled tweezers into grips and followed same testing procedure aforementioned (no sample contact with hands).

VIII. Compression Test

Compression specimens cut from cylindrical syringe molds, 10 mm in diameter and 8-10 mm in height. Used same software and machine as tension test, but switched out tension arms for

compression plates. Strain rate set to 15 mm/min. Cleaned compression plates and calibrated machine with no specimen on bottom plate. Placed specimen on bottom plate and lowered top plate to lowest point on top of sample (samples not perfectly flat cuts). Zeroed stress and force then started compression test method. Software recorded compressive strain, stress, force applied, and area under stress-strain curve. Procedure used for fabricated room temperature, swollen, and cold, swollen samples.

For cold swollen test, cut specimens placed in -20 °C freezer for 20 minutes. Pair of tweezers were cooled in freezer. Cooled tweezers used to transfer cold sample from freezer onto compression plate and began test method aforementioned.

IX. Swelling Test

Cylindrical hydrogel specimens (10 mm diameter, 8-10 mm height) from four different crosslinkers placed on glass sheet in 70 °C oven for 23 hours. Specimens on glass sheet placed in room temperature open to air for 1 hour to equilibrate specimens to room temperature for weighing. Weighed all samples. Placed specimens into individual 20 mL vials with around 10 mL of DI water, and sealed vials. Placed vials in shaded area and let hydrogels swell for 45.5 hours. Took out swollen samples from vials and dried surface water by lightly encasing in Kimwipe and then weighed each specimen. Calculated swelling ratio using Equation 1:

$$Swelling\ ratio = \frac{W_s - W_d}{W_d}$$

Where W_s is weight of swollen hydrogel, and W_d is weight of dried hydrogel.

Swollen samples wrapped in cling wrap to prevent water loss. Within an hour, room temperature and cold compression tests were performed on swollen samples.

Data Analysis and Results

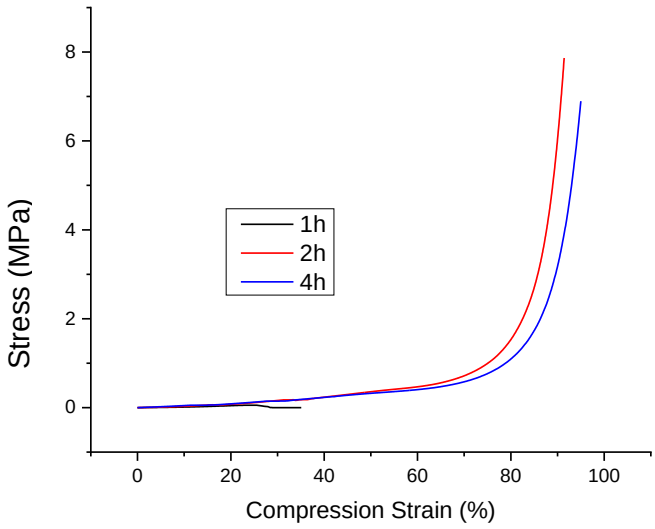
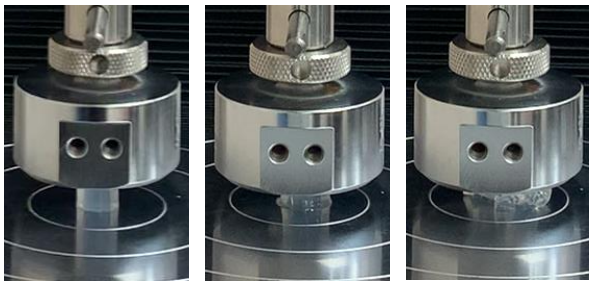


Figure 3. Agar/PAM DN hydrogel compression test for samples exposed to UV light for 1h, 2h, and 4h in presence of MBAA crosslinker.

To observe effect of photoinitiation parameters on mechanical strength of Agar/PAM DN hydrogel, a compression test was performed on three cylindrical molds (Figure 3). All synthesis/testing parameters were held constant besides time molds were exposed to UV light. One mold each was exposed to 1 hour, 2 hours, and 4 hours of UV light for PAM network crosslinking. Sample with only 1 hour of UV light exposure was very weak and fractured at only 25% compression strain and had a max stress of 0.055 MPa.



Figures 4a, b, c (left to right). Agar/PAM DN hydrogel crosslinked with MBAA compression test of 1 h UV exposure at 0 s, 36 s, and 43 s (left to right).

Figures 4a, b, and c exhibits the fracturing of the 1-hour crosslinked Agar/PAM DN hydrogel crosslinked with MBAA. Figure 4a was beginning state of the compression specimen. Figures 4b and 4c are the sample just before and after sample fracture. During compression test, water exuded from hydrogel specimen as compressive strain increased.

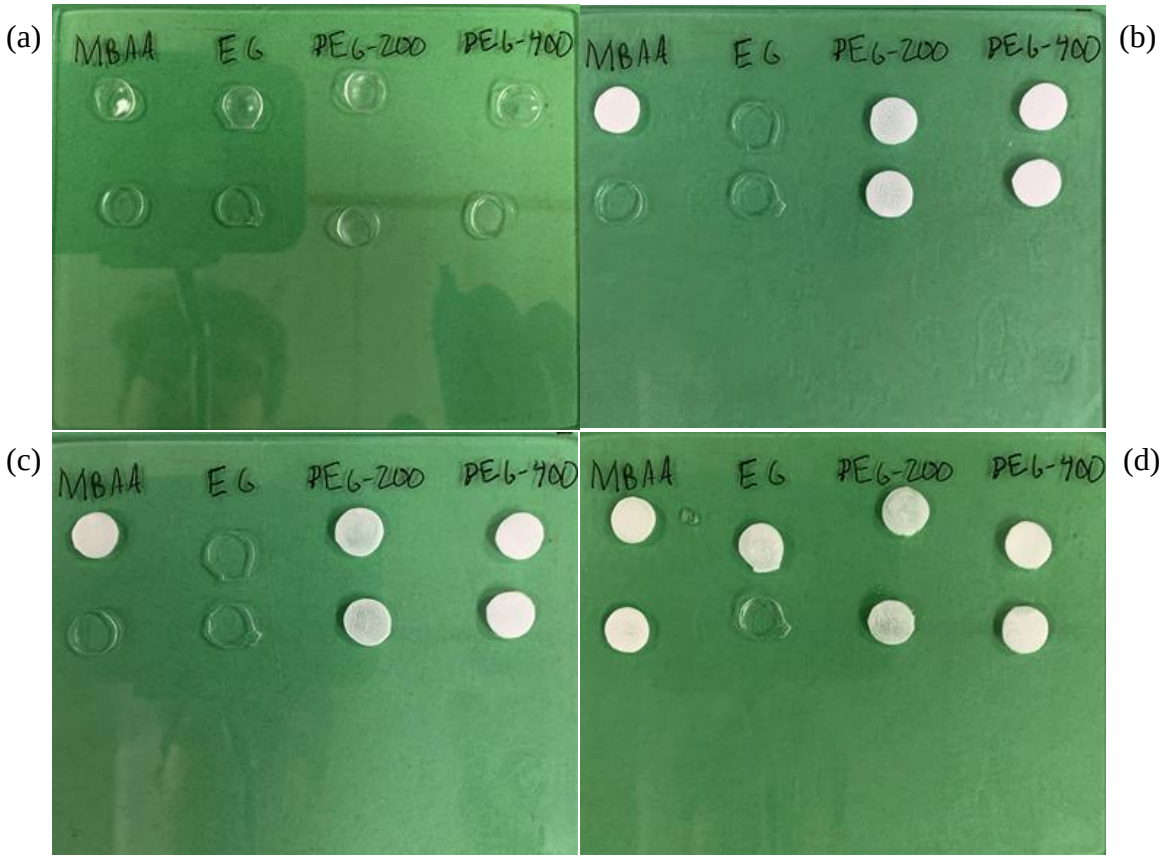
Exposure to 2 hours of UV light experienced the highest stress, 7.87 MPa, and did not fracture (reached 95% compression). The curve for 2 hours does not reach 95% compression

strain (limit for safety of machine) due to inaccurate measurement of sample height and/or beginning set height of compression testing apparatus. Compression % is calculated from an inputted sample height and beginning height of apparatus arm.

Exposure to 4 hours of UV light also experienced 95% compression strain without fracture but a lower maximum stress, 6.89 MPa. Also, exposure for 4 hours resulted in sample becoming opaque, while other samples were transparent.

One hour of UV exposure fractured at low stress and strain due to low crosslinking density of the PAM network. Theoretically, the PAM network can have as many fully crosslinked sites as half of the mols of crosslinker (MBAA) in solution (1.91×10^{-6} mols in this case) or if there are not enough reactive sites on the polymer chains. In this case, the mols of crosslinker MBAA is the limiting factor due to its low 0.03 mol% of the AM monomer. This theoretical number is reliant upon a couple of assumptions: all AM reacts to form PAM chains, no MBAA reacts within PAM chain instead of AM, all MBAA reacts fully with PAM chains at desired reactive site, and all MBAA attached to PAM chains crosslink with each other. All of these assumptions are difficult to control, and take time to allow the reactions to occur. Also, the heterogeneity of the agar network hinders the formation of the PAM network. Too little time resulted in a weaker DN hydrogel.

In the one-pot synthesis method it is challenging to control the PAM crosslinked chain synthesis, due to the simultaneous reactions of the AM monomers and crosslinkers in solution. This is shown by the weaker specimen with four hours of UV exposure, which may be caused by undesired reactions during PAM chain synthesis. One such example is cyclization reactions which is when both ends of the crosslinker react with the forming chain. [11] It has also been reported that increased crosslinking density has an inflection point where it decreases mechanical properties [1].



Figures 5a, b, c and d. 10 mm diameter, 1 mm thick samples of Agar/PAM DN hydrogels crosslinked with different crosslinkers after being in -20 °C freezer for 0, 5, 17, and 30 minutes (a, b, c. and d)

Figures 5 shows freezing time experiment. The formation of ice crystals after only five minutes (Figure 5b) on the hydrogels is most apparent, however, none of the hydrogels were frozen fully (still flexible). After 10 minutes, MBAA samples were nearly fully frozen, but all other PEG samples were easily flexed. After 17 minutes (Figure 5c) the top MBAA sample was fully frozen, EGINA samples were noticeably stiffer, and the PEG_{200}-INA & PEG_{400}-INA samples were both easily bendable. After 30 minutes (Figure 5d), MBAA samples were both frozen and all PEG crosslinkers were still bendable.

The freezing of hydrogels is highly dependent upon the volume of water present within the sample. The same water amount used in fabrication of the hydrogels ensures equal water volume within each sample (although there may be miniscule variations due to loss of water in storage). Also, specimens were taken from areas towards bottom and top of fabricated sheet samples to account for possible difference in water concentration at different locations in hydrogel. This could be due to heterogeneity of the networks, and the potential higher agar network concentration towards bottom of sample since they were cooled standing up. Therefore, another mechanism is dominating the freezing of these hydrogels, and is due to the difference in crosslinkers. The formation of ice crystals on the surface of hydrogels may be due to decreased swelling at lower temperatures. [3] Water within the hydrogel is pushed to the surface, and freezes quickly when their intermolecular bonds are not disrupted by the hydrophilic PAM network.

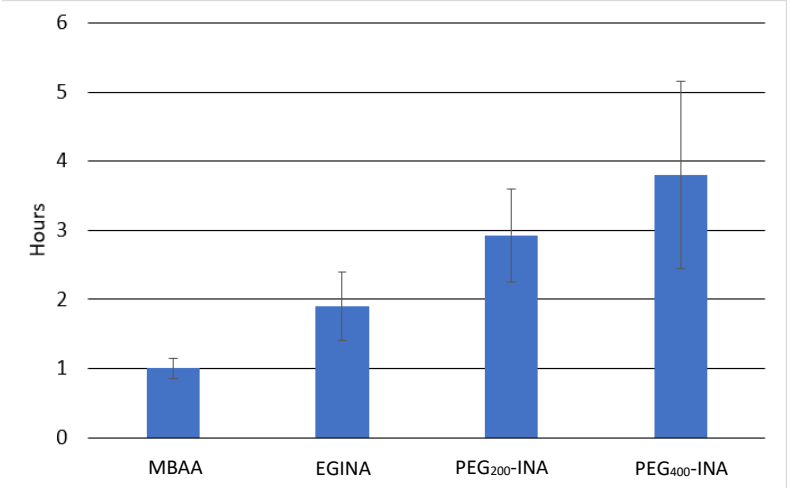
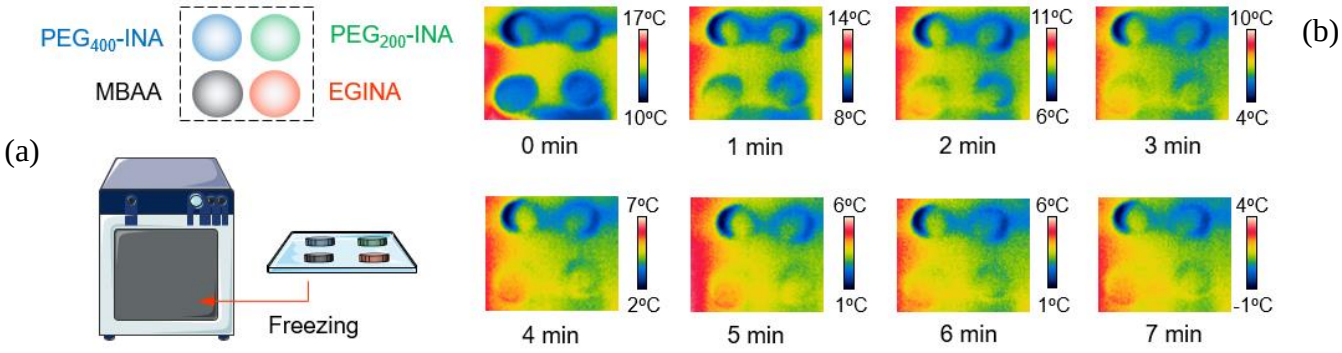


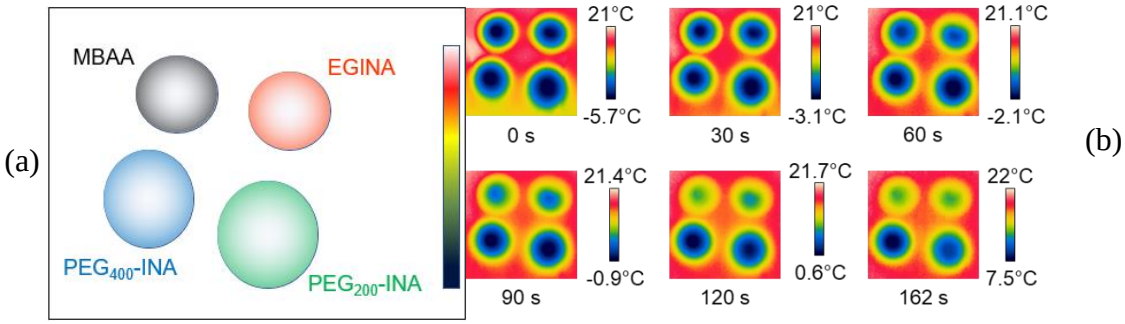
Figure 6. Normalized freezing times of Agar/PAM DN hydrogels with 4 different crosslinkers in -20 °C freezer. Three trials each, standard deviation shown.

Freezing times of the hydrogels in -20 °C freezer with different crosslinkers after three trials were averaged and normalized to MBAA one hour freezing time (Figure 6). Increased PEG repeat units in crosslinker increased observed freezing time.



Figures 7a and b. Diagram of freezing process (a). Thermal images of hydrogel samples in -20 °C freezer (b).

Thermal images of the different crosslink samples in a -20 °C freezer (Figure 7b) show how the samples cooled during the freezing process. The samples were originally colder than the stage due to them being in 2 °C fridge prior, and the glass stage and cardboard beneath was at room temperature. When a sample has the same color as the background, it has cooled to the temperature of the glass staging plate. The MBAA sample equilibrates temperature the quickest after only 2 minutes. The PEG crosslinkers with increasing repeat units show decreasing changes in sample temperature. PEG_{400}-INA shows the least change along with PEG_{200}-INA showing only a slight change. The EGINA sample show a fair amount of change, but takes longer to onset (1 minute) and equilibrate (4 minutes) than the MBAA sample.

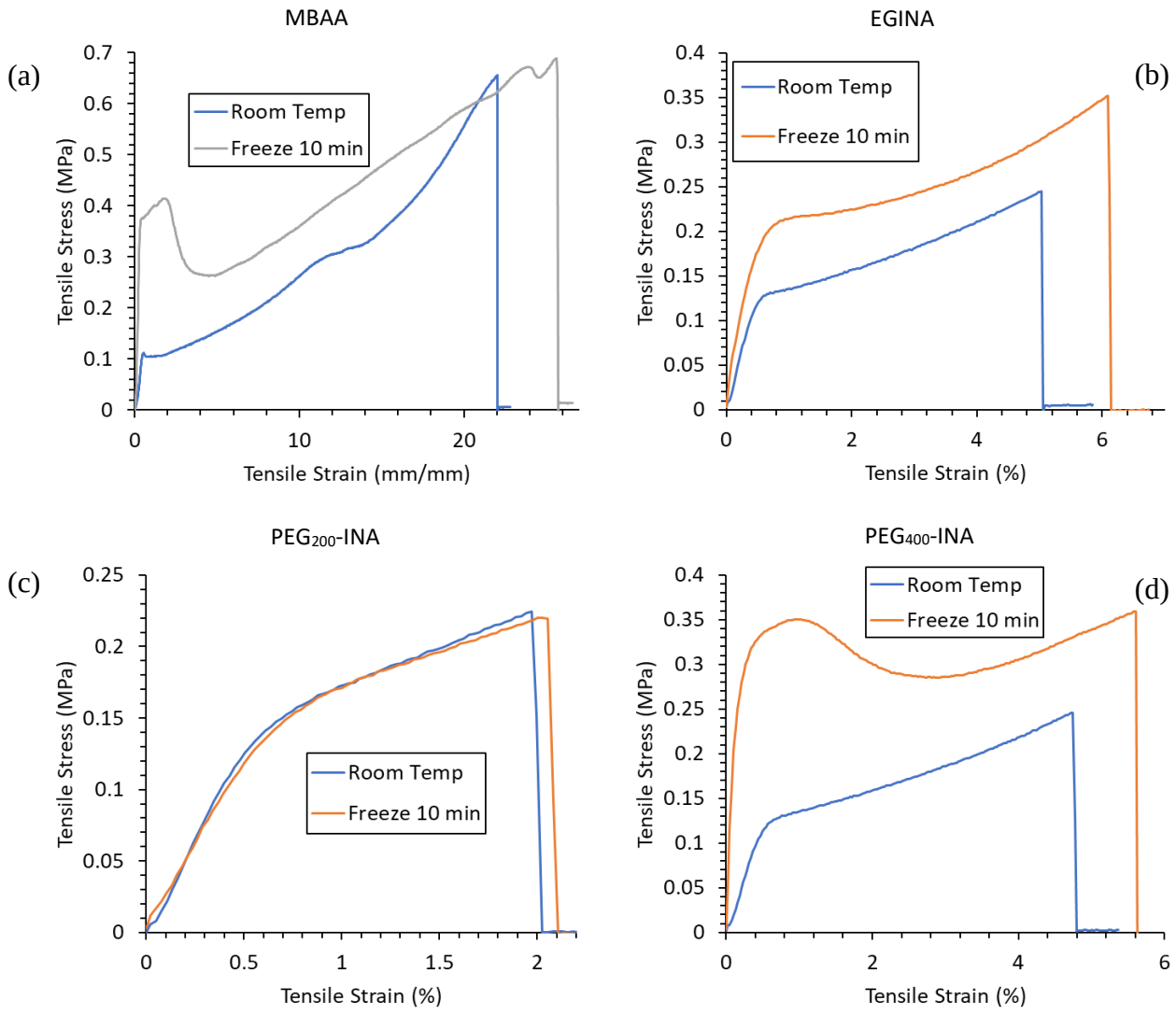


Figures 8a and b. Diagram of thawing process (a). Thermal images of hydrogel samples thawing in room temperature after being removed from -60 °C freezer (b).

Once removed from -60 °C freezer, thermal video was taken of hydrogel samples with different crosslinkers at room temperature (Figure 8b), and observed thawing process. EGINA began noticeably equilibrating to room temperature first at around 34 seconds, and MBAA soon followed at around 35 seconds. Interestingly, MBAA thawed faster than EGINA, noticeable by the smaller cool green center at 2 minutes. PEG_{200}-INA began noticeably changing temperature at 104 seconds, but its rate of temperature change is so small that even after 162 seconds it still has a dark blue center. PEG_{400}-INA sample shows the least amount of change in temperature and served as the low on the temperature scale for the full 2:42 minutes of thawing. It should be noted that the temperature scale minimum does change from -5.7 °C to 7.5 °C from start to finish of experiment. Besides this, what's still noticeable is from MBAA to PEG crosslinkers increasing in molecular weight, heating rate in the samples decreases.

The phenomena observed in Figures 5-8 are most likely caused by the hydrophilic nature of PEG based crosslinkers, which decreases water-water intermolecular forces. Similar to freezing point depression by addition of salt to water to decrease freezing temperature, PEG crosslinked hydrogels showed decreased heat conductivity (Figure 8b) and freezing temperatures (Figure 6). Thermal video of the freezing/thawing process (Figures 7 and 8) showed that PEG based crosslinkers resist temperature change, which is evidence of decreased heat conductivity. Freezing point depression with PEG crosslinkers increased as the molecular weight/length of PEG chain increased. One potential mechanism may be the increased number of oxygen atoms in the crosslinked chain that interact with water in the hydrogel. Another possible mechanism by which heat conductivity and freezing points decreased with increased crosslinker molecular weight, is dispersion forces. Due to the increasing molecular weight from MBAA, EGINA, PEG_{200}-INA, to PEG_{400}-INA crosslinkers (154.2, 348.34, 486.24, 686.24 g/mol respectively) there is greater surface area for interaction between crosslinked chain and water, and greater London dispersion forces.

A third potential mechanism is the physical separation of water clusters by the crosslinked network. To decrease the effect of water crystallization within the hydrogel, it is a goal to create numerous smaller pockets of water crystals rather than larger crystals (increased number of pores). This in theory, would decrease the chance of ice expanding and cracking the molecular structure of either the agar and/or PAM networks. It would also decrease the heat conductivity between water clusters.



Figures 9a, b, c, and d. Tension tests of room temperature specimens, and specimens cooled in -20 °C freezer for 10 minutes with MBAA, EGINA, PEG_{200}-INA, and PEG_{400}-INA crosslinkers (a, b, c, and d)

Samples with different crosslinkers were stretched at 400 mm/min at room temperature and after being in -20 °C freezer for 10 minutes to compare the mechanical properties of the samples at base conditions and lower temperatures. Table 1 below shows the max stress, max strain, Modulus, and area under stress-strain curve for the samples at room temperature and cooled temperature.

Table 1. Tabulated values of max stress, strain, modulus, area under curve, and stress at yield of tension specimens. Modulus is defined as area under curve from 10%-40% of max strain. One trial each.

	Max Stress [MPa]	Max Strain [mm/mm]	Modulus (10%-40%)	Area under curve [kPa]	Stress at Yield [MPa]
MBAA Room	0.656	22.02	0.3792	6571	0.1119
MBAA Freeze	0.689	25.60	2.011	11498	0.4148, 0.6696
EG Room	0.248	5.030	0.3267	840.8	
EG Freeze	0.352	6.090	0.4278	1501	
PEG200 Room	0.224	1.970	0.3168	300.8	
PEG200 Freeze	0.220	2.030	0.2712	309.0	
PEG400 Room	0.246	4.740	0.3005	786.7	
PEG400 Freeze	0.360	5.600	0.9407	1746	0.3543

The MBAA specimen experienced the highest maximum stress and strain at room and cold temperatures (0.656 MPa, 22.02 mm/mm and 0.689 MPa, 25.6 MPa respectively). The next strongest at room temperature was the EGINA, closely followed by PEG_{400}-INA and then the PEG_{200}-INA fracturing at relatively low strain. After being cooled, the next strongest sample is PEG_{400}-INA followed by EGINA and then PEG_{200}-INA.

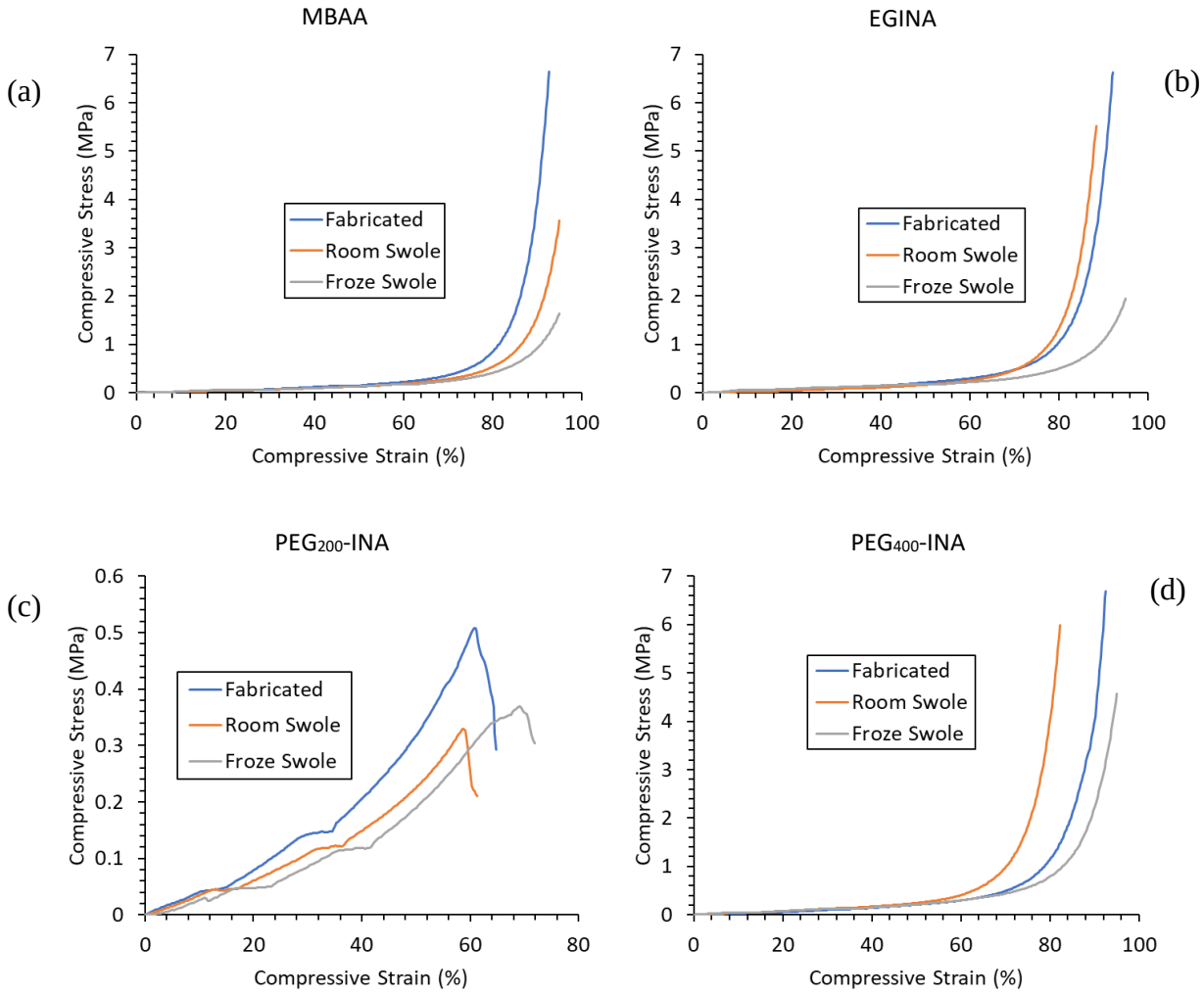
The most interesting observation was that all samples increased in max stress and strain at lower temperatures (besides PEG_{200}-INA crosslinked which had a lower max stress, but higher strain at lower temperature). MBAA exhibited the best mechanical properties (strength, toughness, Modulus) but EGINA and PEG_{400}-INA specimens experienced the greatest increase in maximum strain (16% increase compared to 21% and 18% respectively). PEG_{400}-INA and EGINA specimens also experienced a greater increase in stress at colder temperature (46% and 42% increases compared to only 5% increase respectively).

PEG_{400}-INA at room temperature and MBAA at room and cooled temperatures exhibit yielding. Most notably MBAA at cooled temperature experienced two yielding points at 0.41483 MPa and 0.66962 MPa. One point when sample began to stretch and another near fracture. These yielding behaviors were accompanied by a large increase in the Modulus when the MBAA and PEG_{400}-INA specimens were cooled.

Under tension Chen et al. [6] describes an agar chain pulling-out mechanism. At standard state (fabricated, not stretched, and room temperature) the heterogeneous agar network has a double helical structure in concentrated clusters, entwined with the crosslinked PAM network. At low strain, agar bundles begin unravelling and become more homogenous within the hydrogel which is responsible for yielding behavior. Beyond yielding, the agar network is not fractured but instead slides against each other and the PAM network, which accounts for the near linear increase in stress-strain curve up until near fracture. The sharper increase occurs when the agar chains and PAM network align (strain hardening) and reach their maximum strain before fracture.

The increases in Modulus and yielding behavior at lower temperatures are most likely due to the hardening of the agar network. Such as agar is responsible for the Modulus in room temperature DN gels, this behavior improved at decreased temperature. The MBAA agar network may have fractured which would be indicative of the sharp decrease in stress at yielding point. The chain-pull out mechanism still seems to occur after yield, but at a lower increase of stress with strain than its room-temperature counterpart. This would be due to less entanglement

and alignment at higher strains. While stretching, the specimen absorbs energy and heats up, resulting in a less brittle agar network. This may account for the steadier decrease in stress after yield in PEG_{400}-INA than MBAA exhibited. The PAM and/or agar networks may be less brittle in PEG_{400}-INA due to their cooled temperature during testing being higher than MBAA because of its decreased heat conductivity.



Figures 10a, b, c, and d. Compression test of hydrogel specimens with MBAA, EGINA, PEG_{200}-INA, and PEG_{400}-INA (a, b, c, d) at room temperature fabricated and swollen, as well as swollen samples after being cooled in -20 °C freezer for 20 minutes.

Cylindrical samples with different crosslinkers were tested at fabricated conditions, and swollen at room temperature and after being cooled in -20 °C freezer for 20 minutes. Values of max stress, strain, and % decreases in stress from fabricated or swollen are found Table 2 below.

Table 2. Results of compression test highlighting max strain, max stress, % decrease in max stress from fabricated, and % decrease in stress from swollen for hydrogel specimens with different crosslinkers. One trial each.

	Max Strain [%]	Max Stress [Mpa]	% Decrease in Stress from Fabricated	% Decrease in Stress From Swole
MBAA Reg	92.64	6.638		
MBAA Swole	95.00	3.563	46.32	
MBAA Froze Swole	95.00	1.640	75.29	53.97
EG Reg	92.13	6.634		
EG Swole	88.38	5.515	16.87	
EG Froze Swole	95.00	1.944	70.70	64.76
PEG200 Reg	60.93	0.508		
PEG200 Swole	58.81	0.330	35.12	
PEG200 Froze Swole	69.13	0.369	27.37	-11.94
PEG400 Reg	92.48	6.685		
PEG400 Swole	82.29	5.995	10.32	
PEG400 Froze Swole	95.00	4.572	31.60	23.73

Unlike tension tests, the fabricated PEG_{400}-INA at room temperature exhibited the highest maximum compressive stress, 6.685 MPa, at 95% strain. Second strongest fabricated was MBAA (6.638 MPa, no fracture), and close behind was EGINA (6.634 MPa, no fracture). Fabricated PEG_{200}-INA showed weak mechanical properties fracturing at a max stress of 0.508 MPa and 60.9% compressive strain.

When swollen and tested at room temperature, PEG crosslinkers experienced the smallest decrease in strength when compared to their fabrication standard than did MBAA. PEG_{400}-INA and EGINA displayed the best swollen maximum strength (5.995 MPa and 5.515 MPa respectively) at only 10.3% and 16.9% decreases from their fabricated standards. MBAA crosslinked experienced a staggering 45% decrease in strength when swollen, only achieving 3.563 MPa. None of the swollen samples fractured besides PEG_{200}-INA which decreased 35% in strength from fabrication.

When compared to room temperature swollen standard, PEG_{400}-INA decreased slightly in strength when cooled, 23.7% (31.6% decrease from fabricated). MBAA cooled was second best with a 54% decrease from swollen (75% decrease from fabricated). Then EGINA experienced a large decrease in strength when cooled from swollen, 65%, but was still stronger than swollen MBAA cooled (1.944 MPa and 1.64 MPa). PEG_{200}-INA experienced an increase in strength from swollen when cooled, but was still the weakest by far of samples tested.

These DN hydrogels exhibit anti-swelling properties. The Agar/PAM DN hydrogels with MBAA and PEG based crosslinkers boast swelling ratios of 6.11, 5.65, 5.2, and 5.75 (left to right in Figure 11). Chen et al. [2] even reported Agar/PAM DN hydrogels with swelling as low as 1.3-3.6. This is compared to Jayaramudu et al. [3] that reported a PAM crosslinked MBAA hydrogel with swelling of 11.69.

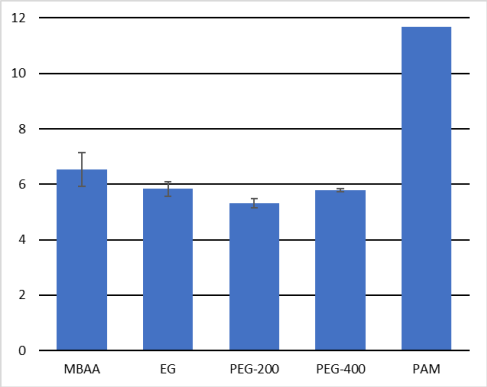


Figure 11. Swelling ratios of Agar/PAM DN hydrogels with MBAA, EGINA, PEG_{200}-INA, and PEG_{400}-INA crosslinks compared to swelling of PAM crosslinked hydrogel (Jayaramudu et al. [3]). Two trials each of reported crosslinkers swelling, and standard deviation shown.

Under compression, the PEG based crosslinked hydrogels strength (besides PEG_{200}-INA) were comparable to MBAA, contrary to tension tests. PEG crosslinkers exhibited lower swelling, however, the 6.9% decrease in swelling ability from MBAA to PEG_{400}-INA does not fully explain the superior performance of PEG_{400}-INA swollen and/or frozen. It could be possible that PEG based crosslinkers created a more brittle PAM network that increased compressive strength (and could also explain the decreased tensile toughness). The superior performance of PEG_{400}-INA while swollen & frozen may be due to the mitigated formation of ice crystals by aforementioned mechanisms. These ice crystals would increase the probability of deformations (potentially increase heterogeneity as well) in the polymer networks, and decrease the hydrogels strength.

Conclusions

Ideal UV light exposure at 365 nm wavelength and 8 W is 2 hours for samples 1 mm thick. Testing at 1 hour proved weak PAM network as sample experienced fracture at low compressive strain. Sample with 4 hours of UV exposure experienced 95% compressive strain (same as 2 hours), however, it didn't achieve as high of a compressive stress. Four hours of exposure also resulted in an opaque sample which may be undesirable in some applications compared to 2 hours of exposure which was transparent. Not enough UV exposure results in less crosslinking density and a less homogenous PAM crosslinked structure leading to decreased mechanical strength. The decrease in mechanical strength from two to four hours may be a result of undesired reactions of the crosslinks creating deformations in the polymer chain, straying away from the ideal cubical structure. Lee et al. [1] also reported a decrease in maximum shear modulus at an increased crosslinking density.

The hydrophilic nature of PEG based crosslinkers decreases water-water intermolecular forces. There are three potential mechanisms that are responsible for PEG based crosslinkers decreased freezing temperatures and heat conductivity. The most dominating mechanisms appear to be increased London dispersion forces with increasing crosslinker molecular weight, and the addition of oxygen molecules in PEG chain. A third mechanism that may affect the thermal properties of the DN hydrogels is the porosity of the PAM network. It is recommended to study the heat conductivity of the hydrogels through DSC experiments to quantitatively study the correlation between molecular weight of crosslinkers and heat conductivity. One example of an experimental procedure for this is outlined by Zhang et al. [4]. Freezing temperatures of the hydrogels should be found to also prove that the freezing point did lower, and that the trends observed were not due only to reduced heat conductivity. To find if porosity and molecular structure of PAM network was affected by PEG crosslinkers, ^{1}H-NMR, SEM, and other such

experiments should be performed. These experiments can hopefully find a trend with the swelling and mechanical properties of PEG hydrogels.

PEG based crosslinkers decreased the swelling of the Agar/PAM DN hydrogel when compared to MBAA. PAM hydrogels are very hydrophilic and exhibit high swelling. [3] Crosslinking of hydrogels has been proven to lower swelling due to increased rigidity of network and decreased space for water between network chains. [5] The high efficiency of PEG crosslinking [1] is seen in the decreased swelling of the Agar/PAM DN hydrogel when compared to the PAM SN hydrogel.

Swelling and low temperatures have lesser effect on PEG based than MBAA crosslinked Agar/PAM DN hydrogels. Indicative by tension test comparisons from room temperature to cooled fabricated samples, PEG_{400}-INA exhibited the best increase in mechanical properties at cooler temperatures. When cooled, MBAA experienced only a 16% in max strain and 5% increase in max stress, while PEG_{400}-INA exhibited 18% and 46% increases in max strain and stress. EGINA also experienced a notable increase in mechanical properties when cooled, with increases of 21% and 42% in max strain and stress. PEG_{400}-INA also exhibited yielding behavior and a high Modulus when cooled, which is indicative of a hydrogels ability to elastically recover [6]. A notable observation is when cooled, the MBAA crosslinked hydrogel exhibited two yielding points. A similar phenomenon is experienced in polyethylene, and was concluded that the first yielding point marks beginning of temporary plastic deformation while the second is associated with permanent plastic deformation. [7] Chen et al. [6] offered the “agar chain pulling-out” mechanism for Agar/PAM hydrogel that exhibited yielding at lower tension strain which was attributed to the double helical agar network bundles lengthening and dragging across the PAM network. A second mechanism may take place at colder temperatures near fracture strain that should be reproduced and studied.

Under compression, PEG_{400}-INA and EGINA mechanical properties were affected less when swollen and cooled than MBAA. When swollen, MBAA decreased 46% in maximum stress while EGINA and PEG_{400}-INA only decreased 17% and 10%. This loss is even greater when swollen and cooled while MBAA, EGINA, and PEG_{400}-INA decreased 75%, 70% and 32% from fabricated. This large difference when swollen and frozen may be attributed to PEG_{400}-INA resistance to temperature change, and mitigation of water crystallization within the network that would have reduce the max strength. More testing should be performed under compression and tension to find the effect of PAM and agar networks on mechanical strength and toughness. It is unknown from results found here whether one network’s performance is more responsible for increases or decreases in mechanical strength. Chen et al. [10] observed experiments on tearing energy that could be used to find the amount of stress each network took on.

Overall, the PEG_{400}-INA crosslinker shows promise as a replacement for MBAA in cold and/or compressive applications. PEG_{400}-INA boasts a lower swelling ratio and temperature effect on mechanical properties. The mol% of PEG_{400}-INA to AM monomer in synthesis may also be optimized in future experiments to increase its strength and toughness, as those parameters and others such as crosslinking efficiency were not questioned in these experiments.

References

[1] Yong Lee, K. Rowley, J.A. Eiselt, P. Moy, E.M. Bouhadir, K. Mooney, D.J. 2000. *Controlling Mechanical and Swelling Properties of Alginate Hydrogels Independently by Cross-Linker Type and Cross-Link Density*. Macromolecules 2000. 33(11): 4291-4294.

[2] Chen, H. Yang, F. Hu, R. Zhang, M. Ren, B. Gong, X. Ma, J. Jian, B. Chen, Q. Zheng, J. 2016. *A comparative study of the mechanical properties of hybrid double-network hydrogels in swollen and as-prepared states*. Journal of Materials Chemistry B. 4:5814-5824.

[3] Jayaramudu, T. Ko, H. Kim, H.C. Kim, J.W. Kim, J. 2019. *Swelling Behavior of Polyacrylamide-Cellulose Nanocrystal Hydrogel: Swelling Kinetics, Temperature, and pH Effects*. Materials. 23(13): 2080.

[4] Zhang, Z. Tan, S. Bao, Z. Wu, Y. Wang, C. 2022. *Thermal Conductivity of PNIPAm Hydrogels and Heat Management as Smart Windows*. Macromolecular Materials and Engineering. DOI: https://doi.org/10.1002/mame.202200566

[5] Pi, M. Qin, S. Wen, S. Wang, Z. Wang, X. Li, M. Lu, H. Meng, Q. Cui, W. Ran, R. 2022. *Rapid Gelation of Tough and Anti-Swelling Hydrogels under Mild Conditions for Underwater Communication*. Advanced Functional Materials. 33(1).

[6] Chen, Q. Zhu, L. Zhao, C. Wang, Q. Zheng, J. 2013. *A Robust, One-Pot Synthesis of Highly Mechanical and Recoverable Double Network Hydrogels Using Thermoreversible Sol-Gel Polysaccharide*. Advanced Materials. 25: 4171-4176.

[7] Brooks, N.W.J. Unwin, A.P. Duckett, R.A. Ward, I.M. 1994. *Double yield points in polyethylene: Structural changes under tensile deformation*. Macromolecular Science. 34(1-2): 29-54.

[8] Morelle, X. Illeperuma, W.R. Tian, K. Bai, R. Suo, Z. Vlassak, J.J. 2018. *Highly Stretchable and Tough Hydrogels below Water Freezing Temperature*. Advanced Materials. 30(35).

[9] Zhou, Y. Wan, C. Yang, Y. Yang, H. Wang, S. Dai, Z. Ji, K. Jiang, H. Chen, X. Long, Y. 2018. *Highly Stretchable, Elastic, and Ionic Conductive Hydrogel for Artificial Soft Electronics*. Advanced Functional Materials. 29(1)

[10] Chen, Q. Zhu, L. Huang, L. Chen, H. Xu K. Tan, Y. Wang, P. Zheng, J. 2014. *Fracture of the Physically Cross-Linked First Network in Hybrid Double Network Hydrogels*. Macromolecules. 47: 2140-2148.

[11] Elliot, J.E. Macdonald, M. Nie, J. Bowman, C.N. 2004. *Structure and swelling of poly(acrylic acid) hydrogels: effect of pH, ionic strength, and dilution on the crosslinked polymer structure*. Polymer. 45(5): 1503-1510.

[12] Chen, H. Chen, Q. Hu, R. Wang, H. Zhang Newby, B. Chang, Y. Zheng, J. 2015. *Mechanically strong hybrid double network hydrogels with antifouling properties*. Journal of Materials Chemistry B. 3:5426-5435.