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Article

Utilizing sorbitol to improve properties of cellulose-based biodegradable hydrogels

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SUMMARY

Hydrogels are commonly used in medicine, pharmaceuticals, and agriculture. Hydrogels absorb water by swelling and re-release this water by diffusion. Therefore, hydrogels that can absorb more water will be more useful in these applications. This study sought to synthesize a biodegradable, cellulose-based hydrogel that is more effective at absorbing and re-releasing water than those produced by current methods. We prepared crosslinkers with the ratios of moles citric acid (CA) to moles sorbitol (S) ranging from 1:6 to 90:1. We selected three crosslinkers (3:1, 10:1, and 54:1) to produce hydrogels with carboxymethyl cellulose and hydroxyethyl cellulose. We tested the compressive strength of both the dry and swollen gels and the tensile strength of the swollen gels to elucidate the gel structure. We hypothesized that a 10:1 ratio of CA to S would produce the most effective hydrogel. We compared the swelling ratios to a gel crosslinked solely with CA. 3:1 gels aided in soil moisture retention the most, revealing that a 3:1 ratio of CA to S produces the most effective gels for water absorption and re-release. Fourier Transform Infrared (FTIR) spectra demonstrated that gel production was successful and that functional groups were the same across all ratios. S improved the properties of hydrogels with different ratios necessary to maximize various parameters. These new hydrogels will be able to better improve plant survival in drought conditions, deliver drugs directly to tissues that need them, and prevent brain injury when applied to helmets.

INTRODUCTION

Over the past decade, carbon dioxide emissions have increased from 397.62 ppm to 422.08 ppm based on yearly averages (1). This has resulted in significant changes in overall global climate, causing prolonged and frequent droughts globally. The agriculture sector is the most drought-sensitive in the economy because high temperatures and low soil moisture lead to decreased crop yields (2). This has caused substantial concerns for countries such as India, where sufficient water for irrigation is unavailable, fertilizer loss is prevalent, and food demand is elevated (3). Direct watering has been employed to combat this but it is not cost effective and a significant amount of water is lost in the process (2). Indonesia has also been gravely affected by these droughts since the country is highly dependent on rice, corn, and soybean crops which do not grow well under these conditions (4).

Hydrogels are a possible solution to these problems caused by droughts. Hydrogels can swell to 200–800 times their original size when submerged in water and slowly release the water obtained by diffusion (3). This makes hydrogels particularly useful in fields such as medicine, pharmaceuticals, horticulture, and agriculture (5). Hydrogels have high water content making them highly biocompatible and allowing them to be introduced into tissues for drug delivery (6). Recent research has shown hydrogels to be effective at preventing brain injury when used in helmets, as swollen hydrogels can absorb some of the force a helmet is subjected to with hydrogels with higher compressive strengths absorbing more force before breaking (7). The two categories of hydrogels are synthetic and natural. Natural hydrogels are biodegradable and biocompatible and include gels made from organic polymers such as starch, cellulose, chitosan, gelatin, or collagen (3). Most traditional synthetic hydrogels are acrylate-based so they are not biodegradable. They degrade slowly and may release toxic chemicals during their degradation. This has pushed research to focus on more environmentally friendly alternatives (8). One of these alternatives is cellulose. Cellulose is a highly favorable option for hydrogel production because it is biodegradable, biocompatible, renewable, mechanically strong, environmentally friendly, and one of the safest materials on earth (5).

A crosslinker is a chemical that links together molecules to improve hydrogel properties. A crosslinker helps form the hydrogel's 3D structure, increasing molecular weight, improving stability, and affecting elasticity, viscosity, and insolubility (5). Crosslinking can be performed with citric acid (CA). CA is widespread, being present in citrus foods, soft drinks, and effervescent salts. It is used in the cleaning and polishing of metals and acts as a key component in the Krebs Cycle (also known as the Citric Acid Cycle) performed in the mitochondria of cells for the production of ATP (9). Heating CA causes it to form a cyclic anhydride which then attacks a hydroxyl group on a cellulose molecule, forming an ester bond (**Figure 1**). This then occurs with the other carboxyl groups on CA, linking the two cellulose polymers (10). Sorbitol (S) is a chemical mainly used in pharmaceuticals to improve molecular stability during freezing, drying, and storage. It has been used in hydrogels as an agent to make the gels more elastic and flexible (11). The abundance of hydroxyl groups on S can allow for CA to react with it and produce a new crosslinking agent that may improve hydrogel properties. While some papers have included S in hydrogels before, the S was added to the reaction mixture with cellulose and CA, making it difficult to determine where the S connected in the gel structure (11,12). Reacting S and CA separately before

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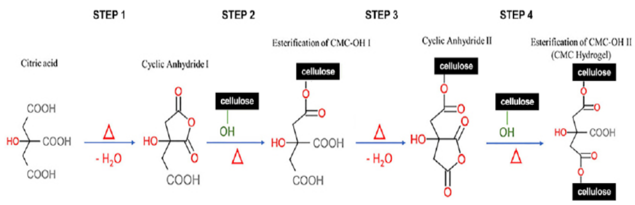

Figure 1: Mechanism for CA crosslinking. When heated, CA loses water to form a cyclic anhydride which attacks an -OH group on cellulose, forming an ester bond. This reaction can repeat for the other two carboxyl groups on CA, linking two cellulose chains together.

adding cellulose would allow for a better understanding of where the S is in the structure.

The objectives of this study were to produce hydrogels more effective at absorbing and re-releasing water than those produced by current methods and to determine the ratio of CA to S that produces these more effective hydrogels. This was achieved by producing gels with varying ratios of CA to S and testing their swelling ratios, compressive strength, and tensile strength, along with applying them to soil in a soil moisture retention test. We hypothesized that a 10:1 ratio of CA to S would produce the most effective hydrogel (13). This ratio would have the S coated in the most carboxyl groups allowing the gel to make the most crosslinked network of cellulose chains while also having enough S to react with a large portion of the CA (**Figure 2**). The 3:1 ratio was expected to perform the worst because we predicted it to link more to itself than the cellulose chains (13). We predicted that the compressive strength would be the same across all ratios as the gels should have similar degrees of crosslinking while tensile strength would be higher for the gels with S than the CA gel because S is known to increase gel elasticity (11). Our results revealed that a 3:1 molar ratio of citric acid to sorbitol produced the gels with the highest swelling ratio which, when applied to soil, aided in moisture retention the most. The significance of this research is that our novel method of synthesizing hydrogels improves on previous methods, enabling them to be more effective at increasing plant survival in drought conditions, delivering drugs directly to tissues, and preventing brain injury when used in helmets.

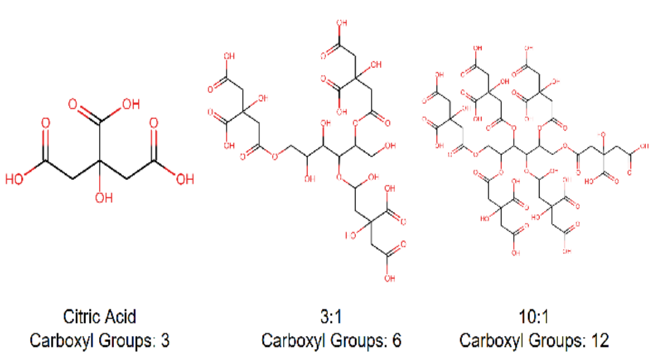

Figure 2: Hypothesized crosslinkers and their carboxyl groups. Citric acid, on the left, only has three carboxyl groups. Reacting citric acid and sorbitol in a 3:1 molar ratio was predicted to produce the middle crosslinker with 6 carboxyl groups. Reacting citric acid and sorbitol in a 10:1 molar ratio was predicted to produce the rightmost crosslinker with 12 carboxyl groups.

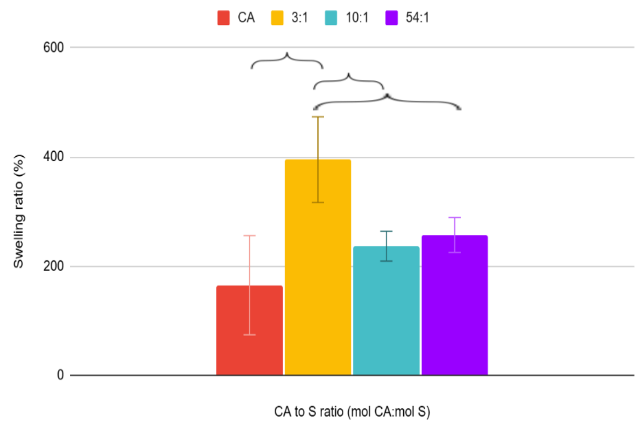

Figure 3: The effect of CA to S ratio on swelling ratio. 5 samples of each ratio were submerged in distilled water for 24 hours to determine swelling ratio (n = 5). 3:1 gels had a significantly higher swelling ratio than the other gels (one-factor ANOVA, $p < 0.05$). } = significant. Data shown as mean ± standard deviation.

RESULTS

We wanted to determine which ratio of citric acid to sorbitol produced the most effective hydrogels at absorbing and re-releasing water. To answer this question, we tested swelling ratios, compressive strengths, and tensile strengths of the gels. We also tested the gels in soil to evaluate their use in agriculture.

Swelling Ratio

We performed swelling ratio tests to determine how effective each hydrogel was at absorbing water. We submerged gels in distilled water for 24 hours and weighed them to determine swelling ratio. The average swelling ratios (n = 5) were 165 % (Standard Deviation (SD) = 90.8 %) for the CA gels, 395 % (SD = 78.5 %) for the 3:1 gels, 237 % (SD = 27.2 %) for the 10:1 gel, and 257 % (SD = 31.9 %) for the 54:1 gels (**Figure 3**). The gels with CA:S ratio of 3:1 had significantly higher swelling ratios compared to all other gels ($p < 0.01$, one factor ANOVA). Gels made with S also had higher average swelling ratios than those crosslinked with CA only.

Compressive Strength of Dry Gels

We tested the compressive strength of the dry gels to elucidate further properties of the gel structure. Less compressive strength would allow the gels to be broken apart easier for increasing surface area for absorption. This test was carried out by measuring the force required to break a dry gel on a scale. The average breaking forces (n = 5) were 197 Newtons (N) (SD = 76.4 N) for the CA gels, 144 N (SD = 14.5 N) for the 3:1 gels, 131 N (SD = 21.5 N) for the 10:1 gels, and 96 N (SD = 8.2 N) for the 54:1 gels (**Figure 4**). CA gels had significantly more compressive strength than 54:1 gels ($p < 0.01$, one-factor ANOVA).

Compressive and Tensile Strength of Wet Gels:

We tested the compressive and tensile strengths of the wet gels to elucidate more of the properties of the gels. These tests demonstrate the extent of crosslinking for each gel. We tested compressive strength by measuring the force

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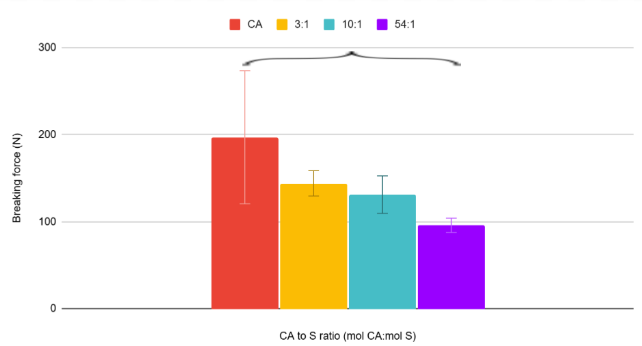

Figure 4: The effect of CA to S ratio on the compressive strength of dry gels. Using a scale, the force required to break dry gels of each ratio was determined (n = 5). The breaking force for the CA gels was significantly higher than that of 54:1 gels (one-factor ANOVA, $p < 0.05$). } = significant. Data shown as mean ± standard deviation.

Time (hr)	Control	CA	3:1	10:1	54:1
0	wet+	wet+	wet+	wet+	wet+
24	wet+	wet+	wet+	wet+	wet+
48	dry	dry	nor	dry	dry

Table 1: Water retention of soil samples treated with different hydrogels over time. Gels were crushed into a powder and mixed with loamy sand soil and the moisture content was observed after 24 and 48 hours (n = 1). Soil was considered wet+ if it had a moisture content of >30%, wet if it had a moisture content of 25–30%, nor if it had a moisture content of 20–25%, dry if it had a moisture content of 15–20%, and dry+ if it had a moisture content of <15%. Soil treated with 3:1 gels retained the most moisture over the 48 hour period.

required to break a swollen gel on a scale. We tested tensile strength by measuring the maximum extension the gels could withstand. The average breaking forces (n = 5) were 12.0 N (SD = 6.41 N) for the CA gels, 13.7 N (SD = 8.66 N) for the 3:1 gels, 8.5 N (SD = 3.01 N) for the 10:1 gels, and 17.3 N (SD = 9.58 N) for the 54:1 gels with no significance between the groups ($p > 0.05$, one-factor ANOVA) (**Figure 5A**). The average maximum extensions (n = 5) were 3.6 mm (SD = 1.7 mm) for the CA gels, 4.2 mm (SD = 2.6 mm) for the 3:1 gels, 5.2 mm (SD = 2.2 mm) for the 10:1 gels, and 2.4 mm (SD = 1.1 mm) for the 54:1 gels with no significance between the groups ($p > 0.05$, one-factor ANOVA) (**Figure 5B**).

Soil Moisture Retention

Despite it being hypothesized that the 3:1 gels would swell the least, the swelling ratio test revealed these gels to be the best performer. That result led us to perform additional functional tests to evaluate its use in agriculture. We did this test by crushing the gels into a powder and mixing it with loamy sand soil in 4 petri dishes (n = 1). The control petri dish did not contain gels. Ten milliliters of distilled water was added to each petri dish. Soil moisture was checked after 24 and 48 hours. All samples remained wet+ (moisture level > 30 %) for the first 24 hours of the experiment, with 3:1 gels becoming normal (nor) (moisture level 20–25%) after 48 hours while the others became dry (moisture level 15–20%) (**Table 1**). This indicates that gels crosslinked with a 3:1 ratio of CA to S are the most effective at improving soil moisture retention.

FTIR Spectra

Fourier Transform Infrared (FTIR) Spectroscopy measures a sample's ability to absorb infrared light, producing a spectrum that allows us to determine the functional groups present in the sample. We produced FTIR spectra to determine the functional groups in the gels and confirm successful gel production. The spectra showed peaks corresponding to carbonyl (~1700 cm^{-1}) and hydroxyl (3200–3700 cm^{-1}) groups which would be expected in the gels, confirming the success of gel production (**Figure 6**). Not only that, but the spectra demonstrated the same peaks, meaning that the gels have the same functional groups.

DISCUSSION

We wanted to determine what ratio of citric acid to sorbitol in the crosslinker produced the most effective gels at water

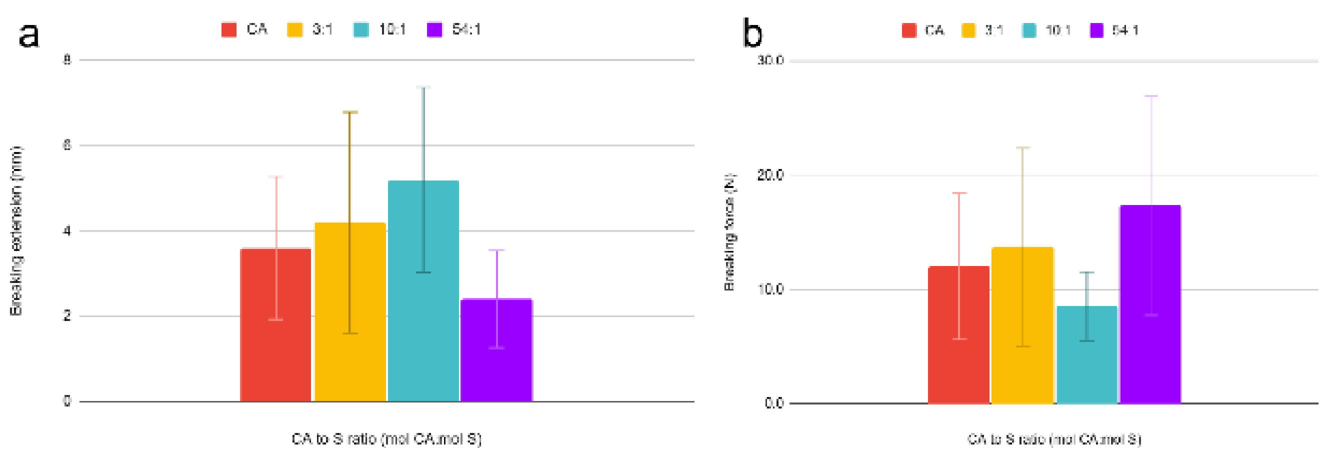

Figure 5: The effect of CA to S ratio on compressive and tensile strength of wet gels. Data for a) tensile strength b) compressive strength. For tensile strength, swollen gels were stretched to determine the maximum extension they could withstand (n = 5). No significance was observed (one-factor ANOVA, $p > 0.05$). Data shown as mean ± standard deviation. For compressive strength, using a scale, the force required to break the swollen gels of each ratio was determined (n = 5). No significance was observed (one-factor ANOVA, $p > 0.05$). Data shown as mean ± standard deviation.

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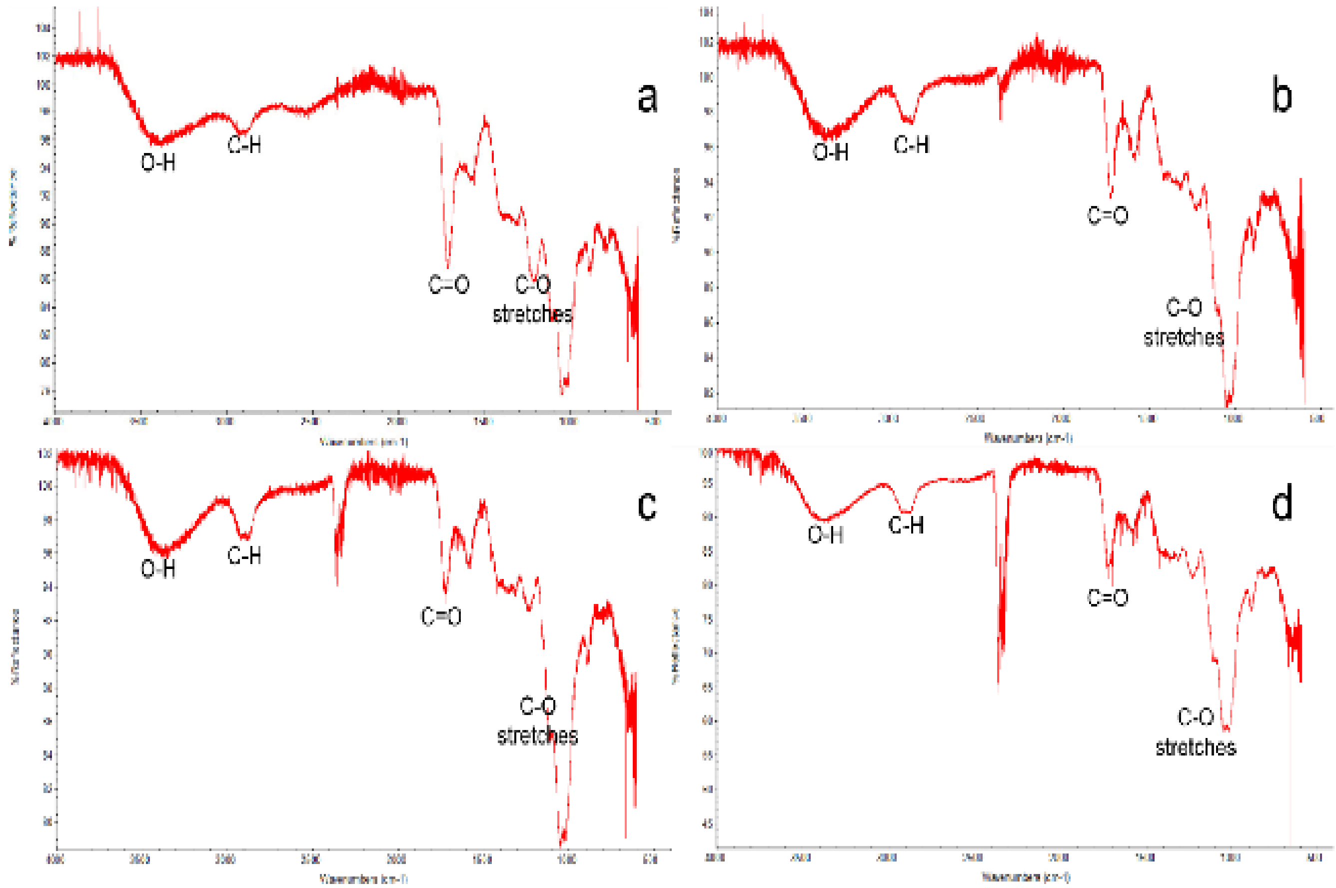

Figure 6: FTIR spectra for hydrogels produced. Spectra for a) CA b) a 3:1 Ratio of CA to S c) a 10:1 Ratio of CA to S d) a 54:1 Ratio of CA to S. Samples were analyzed in an IR spectrometer. Spectra show the same peaks indicating the same functional groups, including hydroxyl and carbonyl groups. Horizontal axis is wavenumbers (cm-1) and vertical axis is % Reflectance.

absorption and re-release. We hypothesized that a 10:1 molar ratio would produce these most effective gels. We tested the gels' swelling ratio, compressive strength, and tensile strength and ran a soil moisture retention test on soils with our gels. We observed that a 3:1 ratio produced the gels with the highest swelling ratio and helped soil retain its moisture the longest.

Most other studies have prepared gels like ours without S and those that use S react the S with both the CA and cellulose chains simultaneously (3,9,11). Our gels differ from those gels as reacting CA and S separately allows us to better understand the structure. If all the components were reacted together, there would be a lot of variation in the size of the crosslinkers connecting the cellulose chains. Reacting the CA and S separately reduces that variation and makes the gels more replicable. The swelling ratios of our gels are lower than those reported by past studies (3,9). But comparing our gels made with sorbitol to those crosslinked with citric acid only, the gels with sorbitol absorb more water, have less compressive strength dry, and do not have significantly more compressive of tensile strength when swollen.

From the swelling ratio results, it was clear that gels crosslinked with a 3:1 ratio of CA to S were the most effective at absorbing water. This does not support our original hypothesis. A possible explanation for this result is that the 3:1 ratio produced larger crosslinkers enabling the gels to swell larger and absorb more water. A possible source of error in this test was differing shapes of the gels. While molds were used to control the shapes of the gels, they were not perfectly uniform causing varying surface area to volume ratios which could impact the swelling ratio. Further testing of the gels in the soil revealed that 3:1 improved soil moisture retention the most. The soil moisture test provides preliminary evidence that the 3:1 hydrogel may improve water retention in an agriculturally relevant test. Further testing can be done with more replicates of the experiment to provide stronger evidence for the usefulness of the gels in agriculture including applying the gels to the soil surrounding plant roots. However, there is clear evidence that a 3:1 ratio of CA to S produces gels that absorb and re-release water most effectively, which could be highly useful in the agricultural field, as they would maintain soil moisture in drought conditions, aiding in plant survival.

Testing the compressive strength of the dry gels revealed that the gels crosslinked with a 54:1 ratio of CA to S had significantly less compressive strength than those crosslinked with just CA. A possible explanation for this result is that S introduced irregularities in the 54:1 gel structure. A possible source of error in this experiment was possible variation in the angle of application of the force causing different pressures to be applied for the same amount of force. The use of replicates helped combat this random error and statistical

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significance means that this variation likely did not affect the results enough to change the outcome. However, 54:1 gels had the most compressive strength when swollen and the least tensile strength, likely meaning they were more tightly crosslinked than the other gels.

This study can be extended in many ways. One possible investigation is applying these gels to plants or helmets to determine their effectiveness in increasing drought resistance and shock absorption, respectively. Another way is by using different carboxylic acids. The use of S allows for a dicarboxylic acid to be used in the gels such as tartaric acid or oxalic acid, as they would have less carboxyl groups, which may end up further improving the hydrogels. Finally, other starches can be substituted for hydroxyethyl cellulose such as tamarind gum or chitosan

Ultimately, using S in the production of cellulose-based biodegradable hydrogels did improve effectiveness of the gels at water absorption and re-release, with the most effective molar ratio of CA to S being 3:1. Selecting different properties in the gels might lead to different ratios being necessary. For example, a 54:1 ratio might be more useful for applications requiring maximum compressive strength when wet. These new, more effective gels can be implemented in many fields, leading to further developments in agriculture, drug delivery, and shock absorption. In agriculture, more effective hydrogels can improve plant survival in drought conditions. In drug delivery, more effective gels can store more medicine to be delivered directly to the tissue in need. And in shock absorption, hydrogels can possibly be more effective at preventing brain injury.

MATERIALS AND METHODS

Preparation of Crosslinkers:

Crosslinkers were prepared with the12 ratios of moles CA to moles S of 90:1, 54:1, 45:1, 10:1, 6:1, 5:1, 3:1, 2:1, 1:1, 1:2, 1:3 1:5, and 1:6. A mass of S (Sigma, Cat# 240850-5G) and mass of CA (Fisher Scientific, Cat# A940-500) based on the molar ratio (see **Table 2**) were dissolved in 100 mL distilled water in a 250 mL beaker and stirred until a clear solution was developed. This solution was placed in an oven at 75°C for 24 hours. This was repeated for each ratio.

Mole Ratio (CA:S)	Mass CA (g)	Mass S (g)
90:1	19.79	0.21
54:1	19.66	0.35
45:1	19.59	0.41
10:1	18.27	1.73
6:1	17.27	2.73
5:1	16.81	3.19
3:1	15.20	4.80
2:1	13.57	6.43
1:1	10.27	9.73
1:2	6.91	13.09
1:3	5.20	14.80
1:5	3.48	16.52
1:6	2.99	17.01

Table 2: Masses used for producing each crosslinker based on CA to S Ratio. Using the molar masses of CA and S, the masses needed of CA and S for each crosslinker were determined. These masses were dissolved in 100 mL of water for preparing crosslinkers.

Preparation of Hydrogels:

This procedure was adapted and modified from a previous study (3). Crosslinkers with ratios of CA to S of 3:1, 10:1 and 54:1 was selected to be used in the gels. One gram of hydroxyethyl cellulose was stirred in 100 mL of distilled water in a 150 mL beaker until a clear solution was obtained (about 1-2 hours). To this solution, 3 g sodium carboxymethylcellulose was slowly added and stirred until the solution became clear and gel-like (about 4 hours). Two grams of CA was dissolved in the mixture with a glass stir rod. This mixture was placed in 3 mL spherical molds and cooked at 75oC until the gel could maintain its shape outside the mold (about 4 hours). This was repeated for each crosslinker selected, mixing in the crosslinkers immediately after they came out of the oven. We then produced FTIR Spectra for the gels using a Nicolet iS 10 FTIR Spectrometer (ThermoFisher Scientific, Cat# IQLAADGAAGFAHDMAPC).

Testing Swelling Ratio:

Each dry gel was massed and had its mass recorded as m_{dry}. The gels were submerged in distilled water for 24 hours. The gels were removed from the water and surface water was removed by lightly tapping the gel with weighing paper. The new mass of each gel was recorded as m_{wet}. This was repeated for five trials of each ratio. Swelling ratio was calculated according to the following formula: $SR (\%) = ((m_{wet} – m_{dry}) / m_{dry}) \times 100$.

Testing Tensile Strength of the Wet Gels:

Unstretched gels' lengths were measured and recorded. Two toothpicks were pushed all the way through the gels' centers. The toothpicks were pulled apart until the gels broke. The maximum length each gel reached was recorded. The maximum extension of the gels was calculated by subtracting the original length of each gel from the maximum length it reached.

Testing Compressive Strength of Dry and Wet Gels:

Dry gels were placed on a tared scale. Force was manually applied to each gel with a metal weight until it broke. The reading on the scale in pounds of force at the breaking point was recorded. This was repeated for five gels of each ratio. This same procedure was then performed for the wet gels. The force needed to break each gel was converted into newtons for data analysis using the following equation: $F (N) = 4.448222 \times F (lbs)$.

Testing Soil Moisture Retention:

Local loamy sand soil was collected, sifted and aliquoted to five petri dishes so that each dish contained 30 g. The soil in each petri dish was obtained from the same source on

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the same day. Gels crosslinked with CA were crushed into a powder using a mortar and pestle and 1 g of this gel powder was mixed into the soil in one of the petri dishes. This was repeated for 3:1, 10:1, and 54:1 gels. One petri dish contained only soil as a control. Ten milliliters of distilled water was added to each petri dish and the moisture levels in the dishes were measured after 24 and 48 hours using a soil moisture meter (Amazon, ASIN B0CTKSHYWP). Soil was considered wet+ if it had a moisture content of >30%, wet if it had a moisture content of 25–30%, nor if it had a moisture content of 20–25%, dry if it had a moisture content of 15–20%, and dry+ if it had a moisture content of <15%. During the testing time, the dishes were in a room at 25°C with the lids removed.

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