

Enhancing the Mechanical Stability of Carboxymethylcellulose Hydrogels through Freeze Crosslinking: A Comparative Study

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By

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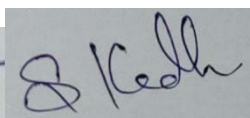
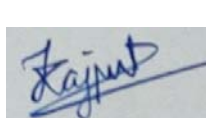
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Certificate

This is to certify that the work incorporated in this **M. Tech Major-Project Report** entitled **“Enhancing the Mechanical Stability of Carboxymethylcellulose Hydrogels through Freeze Crosslinking: A Comparative Study”** submitted by **Mr. Tejas Rajput** to **Academy of Scientific and Innovative Research (AcSIR)** for the fulfilment of the requirements for the award of the Degree of **M. Tech**, embodies original research work under my supervision. We further certify that this work has not been submitted to any other University or Institution in part or full for the award of any degree or diploma. Research material obtained from other sources has been duly acknowledged in the report. Any text, illustration, table etc. used in the report have been duly cited and acknowledged.

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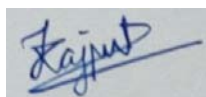
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Thank You

Tejas Rajput

A handwritten signature in blue ink that reads "Rajput" with a horizontal line underneath it.

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1. Abstract

Carboxymethylcellulose (CMC) is a cellulose ether derivative that is highly soluble in water making it a very good material for hydrogel purposes. Though it has poor mechanical strength its mechanical properties can be enhanced with the help of certain crosslinkers. One such crosslinker is citric acid (CA). It has widely been used with CMC to crosslink at high temperatures > 60 . There has also been an attempt to Freeze crosslink CMCF with CA but freeze crosslinking of CMC alone has not been possible yet. Here we have shown one method to freeze crosslink with citric but only if it is prior crosslinked with an acid solution. This technique is a dual crosslinking method to enhance the properties of CMC hydrogel. We have also performed rheology experiments to know the effect of various CA concentration on the properties of the hydrogel. It was found that, less the amount of crosslinker is used, more will be the crosslinking. FEMSEM shows a fibrous network for these dual physically crosslinked hydrogels.

2. Introduction

Hydrogels are a type of material that is referred to as a "soft and wet" substance. Usually, they are made up of a three-dimensional network. Large volumes of water can be held inside the framework of this network of polymer chains. However, traditional hydrogels typically have low strength and poor toughness and thus have a limitation in their practical applications. These issues are often related to the inherent structural heterogeneity, as well as their susceptibility to swelling in water.

Cellulose is a high molecular weight polymer with a linear structure. It is composed of glucose as the repeating units linked together by β -1,4-glycosidic bonds. It is one of the most abundant biopolymers available on this planet. Cellulose is a highly crystalline material with strong inter- and intramolecular hydrogen bonds, which gives it its characteristic high strength and stiffness. However, this also means that cellulose does not readily dissolve or melt in common solvents, which can make it difficult to process and use in certain applications.

To overcome this challenge, researchers have developed various strategies for breaking down the crystalline structure of cellulose to make it more soluble and processable. These strategies include chemical treatments, such as acid hydrolysis or mercerization, and physical treatments, such as mechanical grinding or sonication. Another approach is to use derivatives of cellulose, such as cellulose acetate or cellulose ethers, which have different chemical properties that make them more soluble in certain solvents. Also using eco friendly cross linkers such as citric acid.

Carboxymethyl cellulose (CMC) is one of the most widely used cellulose derivatives (fig. 1). Carboxymethyl cellulose is a semi-flexible, linear, long-chain, water-soluble, anionic, weak, strongly charged polysaccharide that is typically used as a sodium salt (NaCMC). The 2, 3, and 6 hydroxyl groups on the glucose unit of cellulose are partially replaced by the carboxymethyl group to form CMC, a biopolymer. β -D-glucose and β -D-glucopyranose 2-O-(carboxymethyl)-monosodium salt, which are joined by β -1,4-glycosidic linkages, make up the two components of CMC[1]. At the C-2 glucose site, the substitution of a carboxymethyl group for the hydroxyl groups is quite prominent.

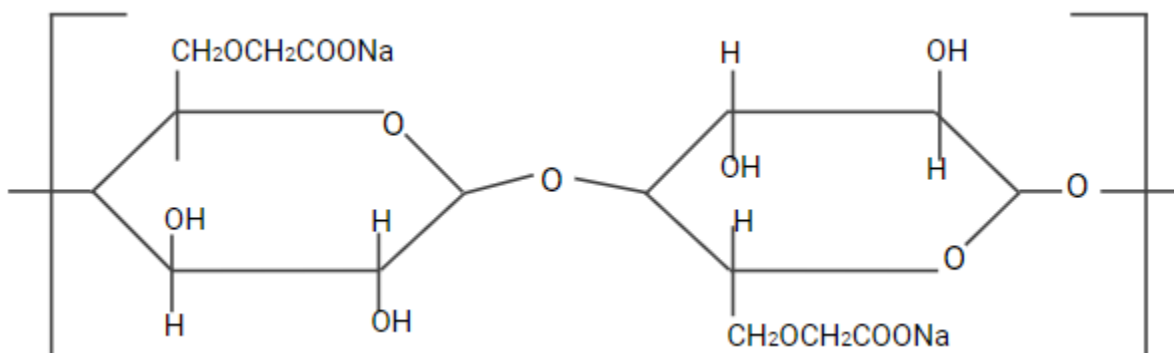


Fig. 1. Chemical structure of CMC

The main reasons why CMC is used are because it has a high viscosity, is environmentally friendly, and is non-allergenic. CMC has water-binding and moisture-sorption capabilities because of the many carboxyl and hydroxyl groups it contains. Due to its inexpensive price, CMC hydrogel offers a wide range of applications and a high water content. It also has good biodegradability. Chemically crosslinked hydrogels may have downsides despite having advantageous structural stability and biocompatibility characteristics.

Alternatives must be discovered for popular chemical crosslinkers including glutaraldehyde, formaldehyde and epichlorohydrin due to their inherent cytotoxicity

CMC has previously been crosslinked with Citric acid[2,3,4,5]. It is chemically crosslinked with citric acid at 60°C or higher temperature. Citric acid will create a cyclic anhydride at high enough temperatures, which will then esterify the hydroxyl groups on the CMC polymer chains. This leads to the formation of chemical crosslinks.

Carboxymethyl cellulose nanofiber (CMCF) has been previously freeze crosslinked with citric acid(CA) solution[6]. But this freeze crosslinking was only possible for the fiber structure of CMCF. When this freeze crosslinking with CA was tried on CMC, it did not show any crosslinking. In CMCF, the freeze crosslinking was possible because of the easy entanglement of fibrous structure. But CMC alone cannot entangle much in the solution and thus it becomes difficult for it to freeze crosslink with CA(fig. 3a).

Using pH-modified carboxymethyl cellulose (CMC) gel we devised a freeze cross-linking technique with citric acid (CA) to produce a dual physically cross-linked hydrogel (fig. 3b). The CMC hydrogel was initially created by adding hydrochloric acid to the CMC solution. Next, an aqueous solution of CA was added to the frozen CMC solution, and thawing the solution further crosslinked the CMC hydrogel.

3. Materials

Carboxymethyl cellulose(CMC) of high viscosity was purchased from s.d.fine-chem Ltd. with Degree of substitution of NLT 0.4, Hydrochloric acid (HCl) 35-38% LR (M.W. 36.46) was purchased from Thomas Bakers, Citric acid (CA) Monohydrate extrapure AR was purchased from srlchem.

All the materials were used as it is without any chemical treatment prior to the experiment.

4. Experiment

4.1. Preparation of Freeze crosslinked CMC hydrogels

The 8wt% CMC solution was first prepared in Deionized(DI) water. It was kept in a vacuum oven at normal temperature to remove the air bubbles from the solution. 2g of this CMC Solution was taken in a glass vial. 2 ml of 1 N HCL was added on top of the CMC solution and it was kept as it is for 24 hours. As CMC is pH responsive in nature it will form gel in the acidic condition. After 24 hours of gel formation the acid solution was removed from the top of the hydrogel. The hydrogel was then kept at -25 °C in the freezer for 24 hours. After that 4 different CA (0.5, 1, 2 and 3)M solutions of 2 ml were added on top of the frozen hydrogel and kept for thawing at -4 °C for the next 24 hours. Then it was kept at room temperature to melt the ice and remove the excess CA solution from top of the hydrogel. It was then washed multiple times with DI water to remove any crosslinker left from the surface of the hydrogel.

4.2. Water Absorption Study:

Hydrogel samples were given several washings with DI water to remove any acid residue. Samples were immersed in DI water for water absorption study. Weight was recorded after every 24 hours. After being taken out of the water, the surface of the sample was carefully wiped off with tissue paper.

%water absorption was calculated according to the given formula below:

$$\% \text{water absorption} = (W_s - W_i) / w_i \times 100$$

Where W_s and W_i are the swollen hydrogel weight and as prepared sample weight respectively.

4.3. Rheology

An ARES G2 rheometer (TA Instruments) with parallel-plate geometry was used to study the rheology of hydrogels. Each hydrogel had a cylindrical shape, measuring 25 mm in diameter and 2 mm in thickness. The dynamic storage modulus (G') of each hydrogel was measured using a dynamic frequency sweep test from 0.1 to 100 rad/s, with a 1% strain rate found to be in the linear viscoelastic range for each type of hydrogel created. All measurements were conducted at a constant temperature of 25°C.

4.4. FESEM

To create aerogels, all of the samples were freeze-dried after being swelled in DI water to establish equilibrium. Field emission scanning electron microscopy (FE-SEM; Hitachi

S-4200) was then used to examine these aerogels. Before SEM imaging, samples were sputter-coated with gold to prevent charging.

5. Gelation Mechanism

CMC is an anionic water-soluble polymer. The formation of the gel is driven by lowering the pH of the CMC solution by adding an 1 N HCl solution on top of the gel and letting it stay for 24 hours.

Ionic strength and solvent pH affect the electrostatic and hydrophobic interactions that play a role in determining the flow characteristics of ionic polysaccharides[7].

In particular at high concentrations, lowering the pH by adding HCl reduces the charge density of the CMC chains and promotes gel strength[8,9]. In order to enhance hydrogen bonding between the polymer chains, carboxyl groups in CMC are protonated after soaking in acid solution[10]. As a result, the CMC molecular chain does not continue to grow, and CMC molecules gather together to form the hydrogen bond. This causes its chain dimensions to decrease[11] and encourages the development of multi-chain aggregates[12]. The gel is created as a result. When a portion of the carboxyl groups are still in the form of $-\text{COONa}$, it is expected that softer gels with fewer hydrogen bonds will form.

Fig.2. explains the process of dual crosslinking of CMC. When this CMC gel is frozen, a freeze-concentrated CMC is created around the ice crystals, isolating the CMC chains from the ice crystals. The concentrated CMC has a lower freezing point than ice[6]. Therefore, when aqueous CA is introduced into the frozen CMC gel, the interaction between concentrated CMCs and CA takes place and we get freeze crosslinked hydrogel (fig.3).

Many of the cellulose molecular chains in CMC are hydrogen-bonded and feature reactive carboxy and hydroxy groups. During preparation, these reactive groups' density in CMC may rise by freeze concentration, resulting in the formation of multi-hydrogen-bonded clusters.

Fig.3a. shows that a hydrogel was not formed even after freeze crosslinking CMC with CA. Fig. 3b. shows a single crosslinked CMC hydrogel which was crosslinked by lowering the pH of the solution. The hydrogel formed was transparent and stable. Fig. 3c shows an opaque hydrogel which shows that it is further cross linked with CA. This hydrogel was freeze crosslinked with CA after forming a gel with HCl.

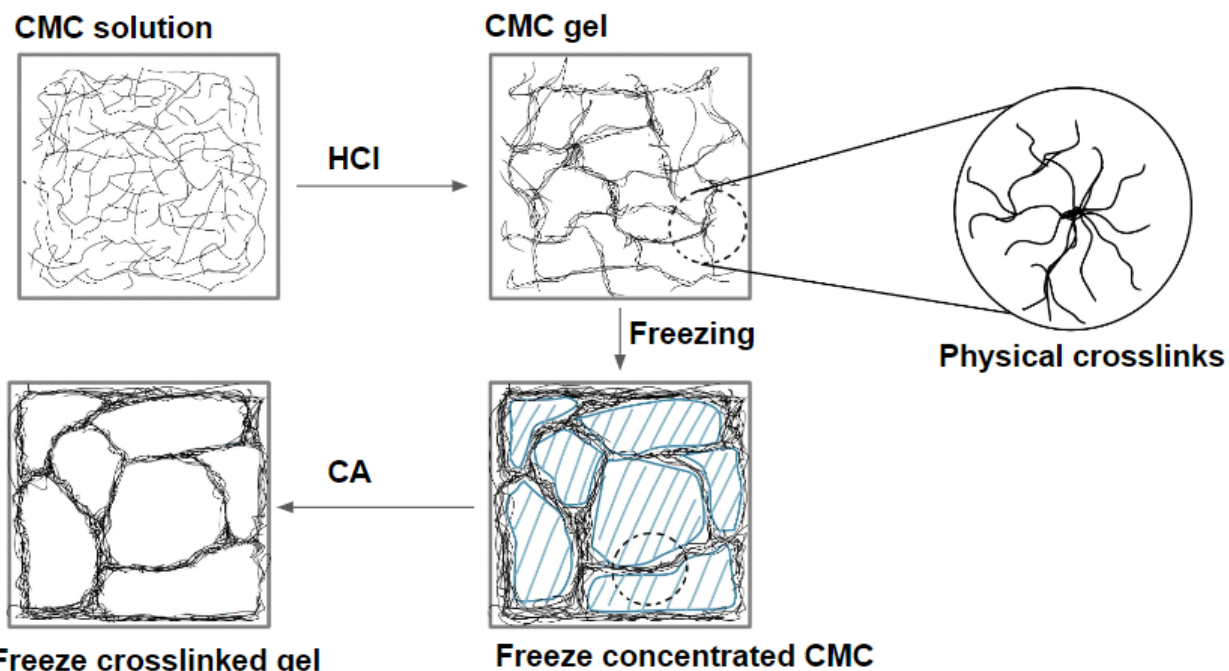


Fig.2. series of schematic illustrations demonstrating the step-by-step preparation of Dual crosslinked CMC hydrogels.

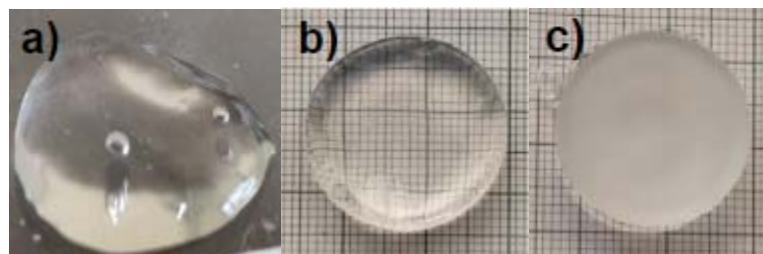


Fig.3. a) Freeze crosslinked CMC gel, b) Single pH crosslinked hydrogel c) Dual crosslinked CMC hydrogel

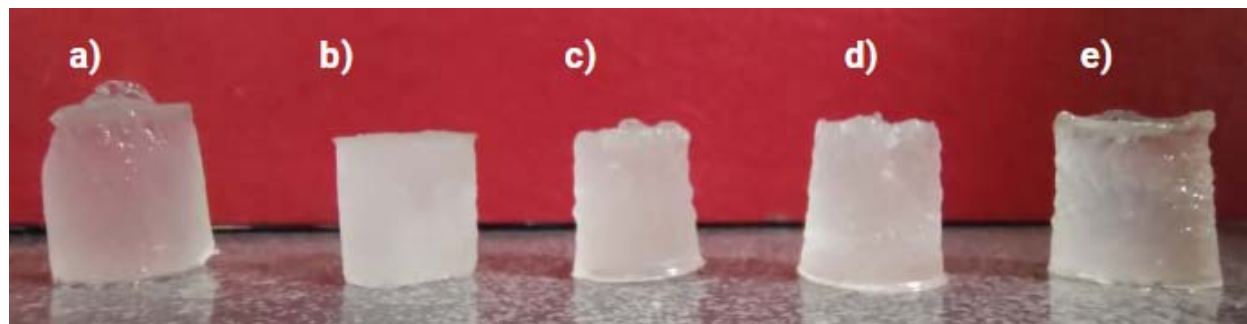


Fig.4. Images of a) CMC_HCL, b) CMC_HCL_0.5M_CA, c) CMC_HCL_1M_CA, d) CMC_HCL_2M_CA, e) CMC_HCL_3M_CA

Fig. 4. Shows all the hydrogels formed from CMC with the help of HCl(fig 4.a) to the dual crosslinked CA hydrogels with varying concentrations (0.5 to 3M). Opacity of the dual crosslinked hydrogel was more than that of the single crosslinked hydrogel which

proves that the hydrogel is getting crosslinked with a freeze crosslinking mechanism. Also the opacity decreases with an increase in the concentration of CA used.

6. Results

Water absorption

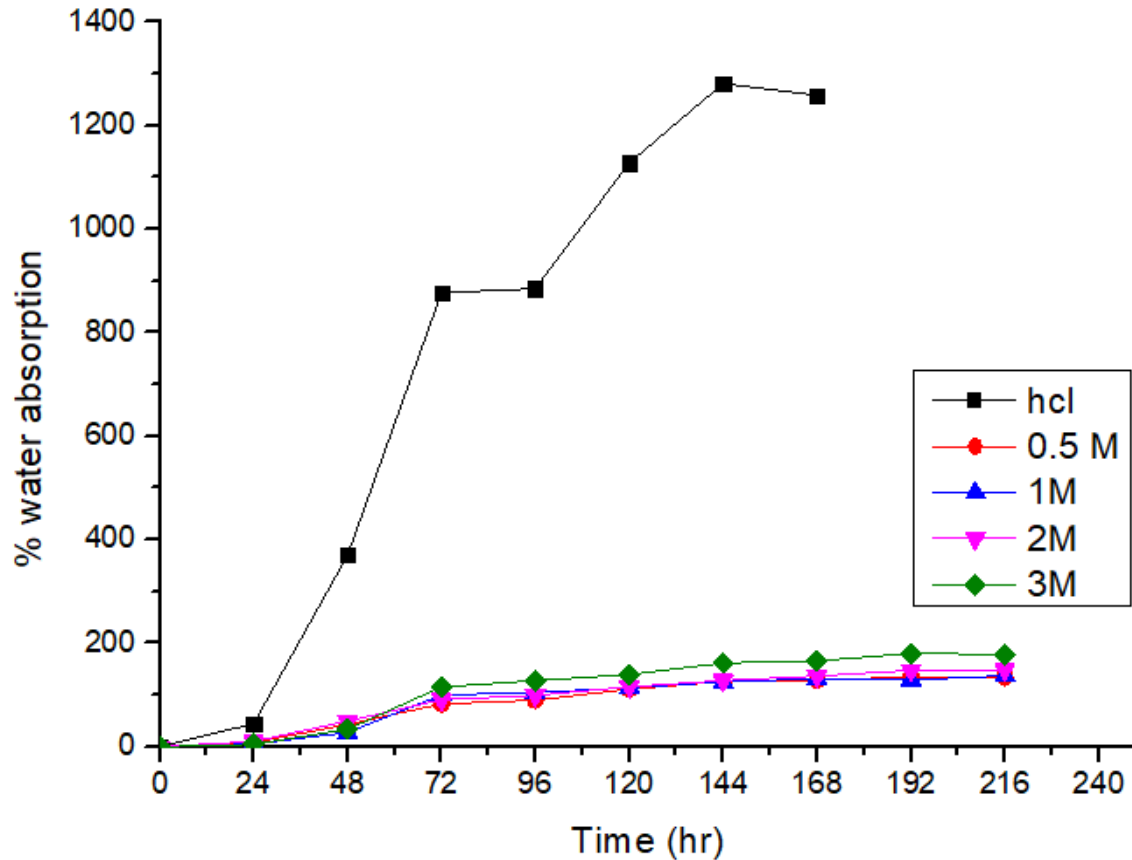


Fig. 5. %water absorption of Hydrogels.

Fig.5. shows the water absorption graph for hydrogels. Highest water absorption was done by single crosslinked CMC hydrogel which was physically crosslinked with 1 N HCl solution. It absorbed 1257% of water. Next Comes the Dual crosslinked Hydrogel with 0.5M CA, 1M CA, 2M CA and 3M CA had a water absorption of 134.5%, 137.4%, 148.9% and 178.8 % respectively. This plot indicating reduced water absorption suggests that dual crosslinking is taking place in the hydrogels. As the concentration of CA increases, one would expect that the crosslinking should increase and water absorption should decrease. Interestingly, with increasing concentration of CA, the water absorption is actually increasing. CA has multiple carboxy groups. When more CA is used, it is likely that all carboxyl groups may not be involved in the crosslinking reaction. This can lead to increase in free carboxyl groups which can lead to increased water uptake of the hydrogels.

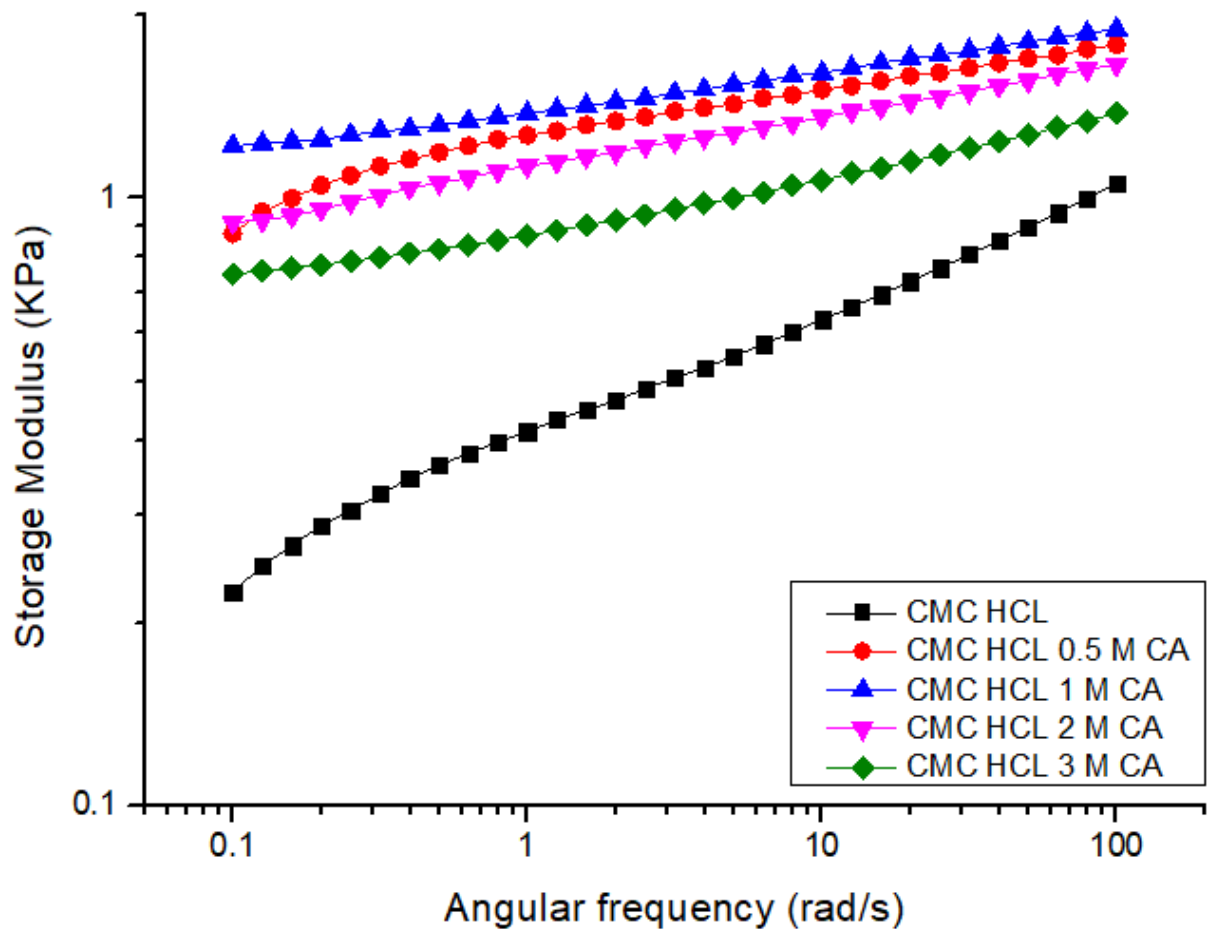


Fig. 6. Variation of storage modulus G' with angular frequency at 25 °C for hydrogels

Fig. 6. and fig. 7. presents storage modulus (G') and loss modulus (G'') of 8% (w/w) crosslinked CMC hydrogels as a function of angular frequency ranging from 0.1 to 100 rad/s at 1% strain. Within the non-destructive LVR zone, the frequency-dependent test assesses the crosslinked hydrogel network's time-dependent viscoelastic capabilities. The low frequency denotes long-term behavior like settling, while the high frequency denotes short-term behavior like mixing and extruding.

Due to the crosslinked hydrogel 3D structure, all hydrogels show an increase in G' at higher frequencies, showing a "solid-like" behavior predominating the gel stability. Single crosslinked hydrogel had lower G' compared to the dual crosslinked hydrogel. 0.5 M and 1 M CA crosslinked CMC hydrogel had the highest modulus of all. G' decreases when the molarity of the CA used is more than 1 M as can be seen in the case of hydrogels with 2 M and 3 M CA.

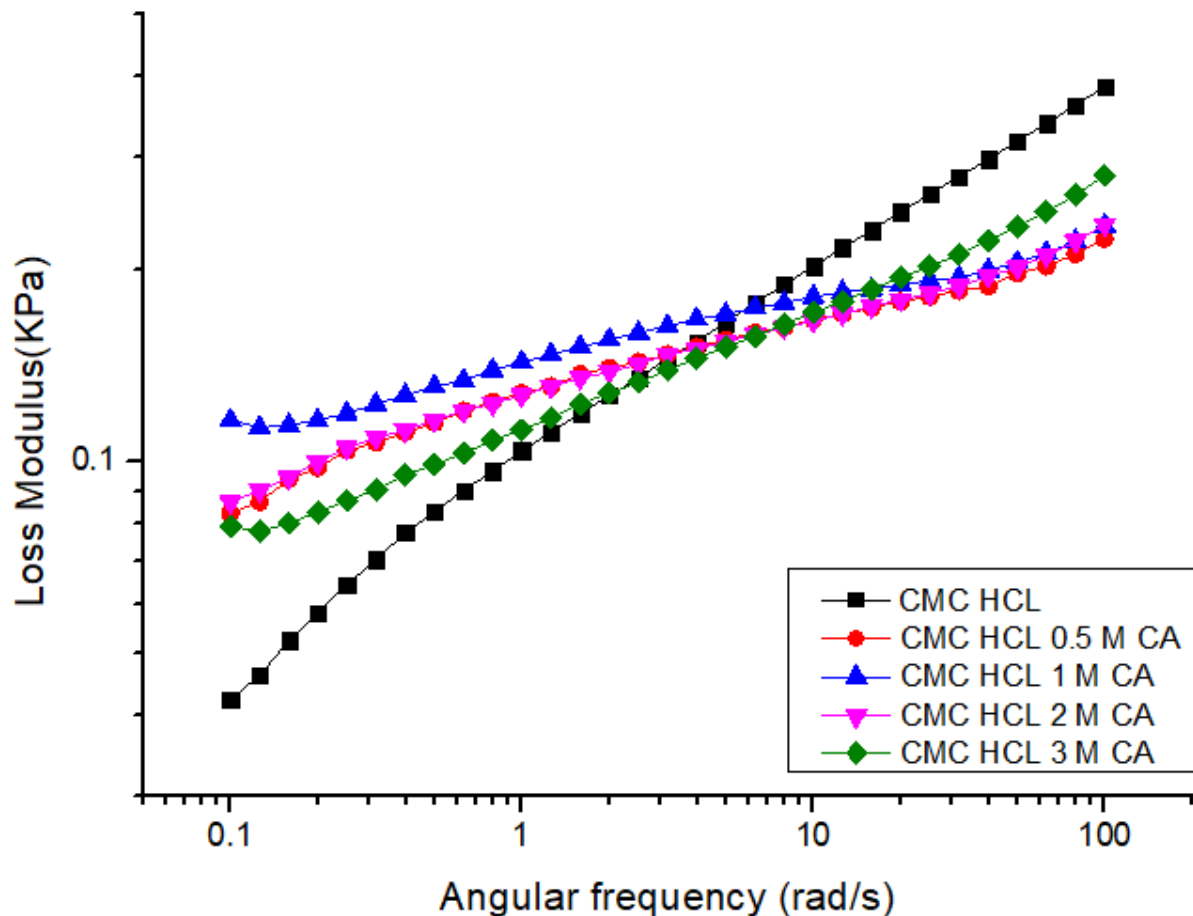


Fig. 7. Variation of loss modulus G'' with angular frequency at 25 °C for hydrogels

Loss modulus G'' for the hydrogels is shown in fig. 7. Over the entire frequency range under study, the G' values for all dual crosslinked hydrogels are over 0.6 KPa, whereas the G'' values for all dual crosslinked hydrogels are less than 0.3 KPa. As a result, in all instances of dual crosslinked hydrogels G' is significantly larger than G'' , demonstrating the hydrogels have high degree of elasticity. In fact, there is no evident physical damage or gel flow when we pick up the dual crosslinked hydrogels by hand or with tweezers. Although the G' was above G'' for single HCl crosslinked hydrogels, the separation was not that much as compared to dual crosslinked hydrogels and was therefore very soft in nature and difficult to handle.

The overall trend shows that dual crosslinked hydrogels have higher storage modulus than single crosslinked hydrogels. Also as the concentration of the crosslinker is increased, the storage modulus decreases. This may be due to the excess citric acid available which can hold more water in the hydrogel which might have increased the flexibility in the chains and decreased the storage modulus. The prevailing consensus is that as water molecules fill up a hydrogel, its elastic ability slowly deteriorates.

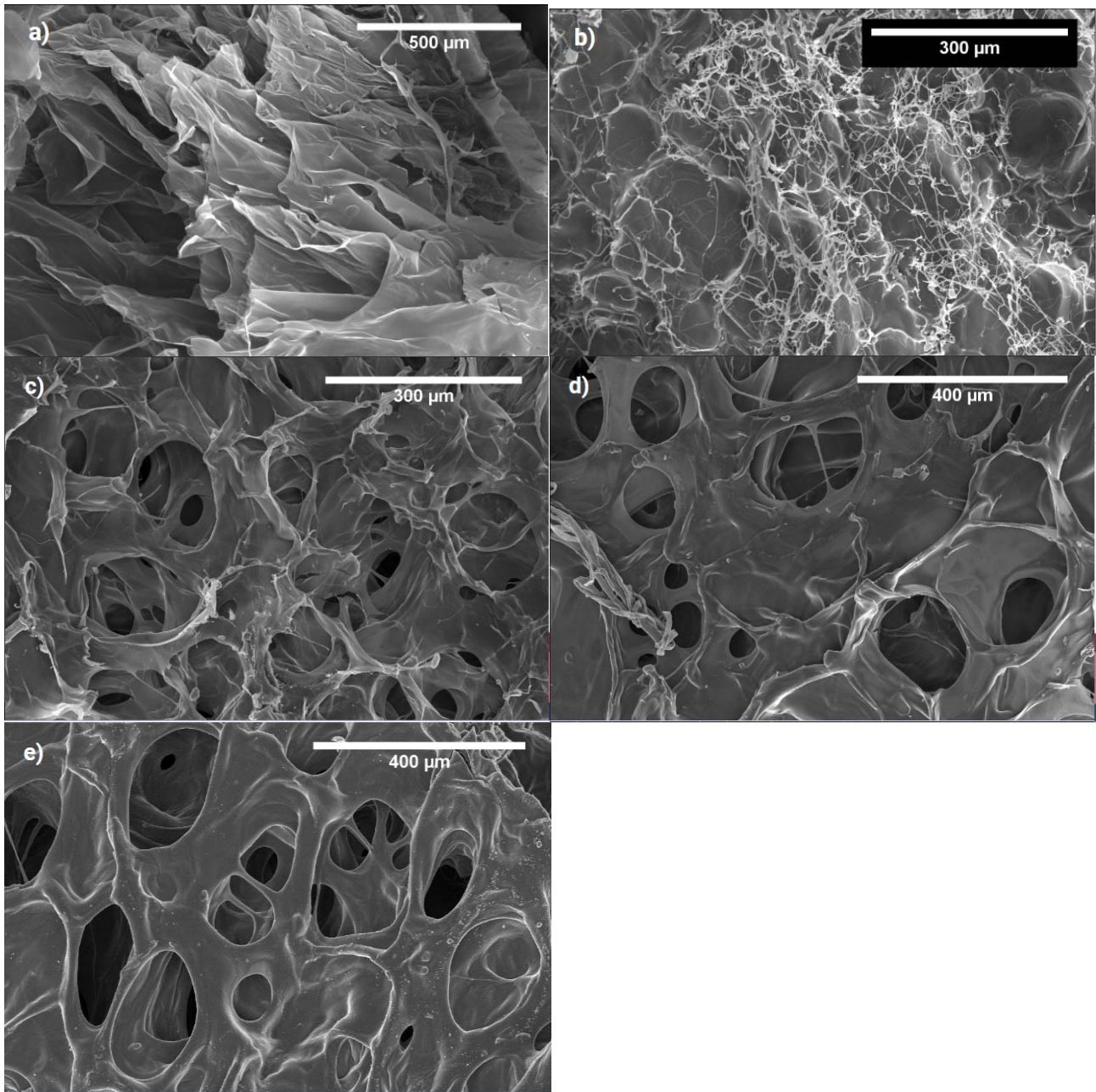


Fig. 8. FESEM images of CMC Aerogels a) CMC_HCL, b) CMC_HCL_0.5M_CA, c) CMC_HCL_1M_CA, d) CMC_HCL_2M_CA, e) CMC_HCL_3M_CA

Fig.8. Shows the FESEM images of aerogel of all 5 compositions of CMC hydrogels. It can be seen that single crosslinked hydrogel is highly porous in nature with surface texture similar to that of films(fig. 8a). Fig. 8e. shows somewhat aggregations on the surface for hydrogel with 3M CA crosslinker. This aggregation keeps on increasing as the concentration of CA is reduced from 3 to 0.5 M. It seems some fibers are highly entangled to each other as the concentration of CA is decreased. For the hydrogel with 0.5 M CA, the surface seems to have a fibrous network with each other and also no pores are seen on the surface of the gel. This effect is supposed to take place because of the varying concentration of CA and as the CA concentration is increased it will hold more water into the hydrogel and increase the free volume in between the CMC chains decreasing the aggregations of chains.

7. Conclusion

CMCF can easily form a freeze crosslinked hydrogel with CA which could not be achieved with CMC alone. We have shown here that pH responsive gel will act as a mediator to further freeze crosslink it with CA. Lower pH will decrease the charge density on the CMC polymer and will increase the hydrogen bonding which will in turn increase the multi chain aggregates. This will give enough opportunity for CA to form crosslinks in the freeze concentrated region which will again increase the aggregations and entanglement of the chains giving a high-strength dual crosslinked hydrogel.

Contrary to expectations, with increasing concentration of CA, the water absorption increased and storage modulus decreased. CA has multiple carboxy groups. When more CA is used, it is likely that all carboxyl groups may not be involved in the crosslinking reaction. This can lead to increase in free carboxyl groups which can lead to increased water uptake of the hydrogels. This has also probably led to lower storage modulus for dual crosslinked hydrogels with higher concentration of CA. Nonetheless dual crosslinked hydrogels had higher storage modulus than the single crosslinked hydrogels and the modulus varied with the varying concentration of CA. In summary, we have shown a facile approach to making dual crosslinked hydrogels and this investigation has provided some insights to tune the water uptake and mechanical properties of carboxymethyl cellulose hydrogels as desired for specific applications.

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