

**PREPARATION AND CHARACTERIZATION OF  
CITRIC ACID CROSS-LINKED  
CARBOXYMETHYLCELLULOSE HYDROGEL**

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**UNIVERSITI SAINS MALAYSIA**

**2021**

**PREPARATION AND CHARACTERIZATION OF  
CITRIC ACID CROSS-LINKED  
CARBOXYMETHYLCELLULOSE HYDROGEL**

**by**

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**Thesis submitted in partial fulfillment of the requirement for the degree of**

**Bachelor of Chemical Engineering**

**July 2021**

## ACKNOWLEDGEMENT

The thesis was done as a partial fulfillment in obtaining the Bachelor of Engineering (Honours) in Chemical Engineering. The thesis was completed with all the support and encouragement from my supervisor, family, and friends.

First and foremost, I would like to grab the chance to express my gratitude to my supervisor, Dr. Masrina Mohd Nadzir, the lecturer of School of Chemical Engineering at Universiti Sains Malaysia for providing me the opportunity to carry out the research for the preparation and characterization of citric acid cross-linked carboxymethyl cellulose hydrogel. Many thanks for her willingness to discuss the research problems, give constructive advice based on my progress, and keeping me on track with this research. Her invaluable guidance, motivation, and willingness have deeply inspired me in analyzing and interpreting the theoretical studies from the works of literature. Besides, I would like to express my appreciation to the School of Chemical Engineering, Universiti Sains Malaysia for allowing the final year students to be exposed to the research works and to enhance our soft skills. I would like to thank the technicians and post-graduate students in lab who willing to share their knowledge and thoughts about the experiment when I approached them. I appreciate their effort in demonstrating the procedure to operate the equipment in laboratory.

Lastly, I would like to extend my gratitude towards my family for their support, love, care, and sacrifices for educating and preparing me for my tertiary education. Once again, I would like to thank all the people, including those whom I might have missed out, and my friends who have helped me directly or indirectly. Their contributions are very much appreciated.

TAN KAI QI

July 2021

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## LIST OF SYMBOLS

<b>Symbol</b>	<b>Description</b>	<b>Unit</b>
$W_d$	Constant weight of dried hydrogel sample	g
$W_f$	Weight of swollen hydrogel sample	g
$W_i$	Initial weight of dried hydrogel sample	g

## LIST OF ABBREVIATIONS

BC	Bacteria cellulose
CMC	Carboxymethyl cellulose
3D	Three-dimensional
APTMS	3-Aminopropyltrimethoxysilane
BET	Brunauer–Emmett–Teller
BJH	Barrett, Joyner, and Halenda
CGG	Cationic guar gum
COO <sup>-</sup>	Carboxylic
COOH	Carboxyl
Cr (VI)	Chromium ion
DI	Deionized water
DVS	Divinyl sulphone
EDA	Ethylenediamine
Fe	Iron
H <sup>+</sup>	Hydrogen ion
HEC	Hydroxyethyl cellulose
IUPAC	International Union of Pure and Applied Chemistry
m/m	Molar ratio
MB	Methylene blue
MIONs	Magnetic iron oxide nanoparticles
MO	Methyl orange
NaCMC	Sodium carboxymethyl cellulose
NaIO <sub>4</sub>	Sodium periodate

OH <sup>-</sup>	Hydroxylic
OH	Hydroxyl
PEG	Polyethylene glycol
PLA	Polylactic acid
PNIPAM	Poly(N-isopropyl acrylamide)
PVA	Polyvinyl alcohol
RhB	Rohdamin-B
SAP	Superabsorbent polymer
SEM	Scanning Electron Microscopy
w/v	Weight-to-volume ratio
Zr(IV)	Zirconium ion
ZrOCl <sub>2</sub> ·8H <sub>2</sub> O	Zirconium oxychloride octahydrate

**PENYEDIAAN DAN KARAKTERISASI HIDROGEL  
CARBOXYMETHYLCELLULOSE BERANGKAI SILANG ASID SITRIK**

**ABSTRAK**

Hidrogel adalah rangkaian rantai polimer fleksibel yang mengandung air yang berangkai secara fizikal atau kimia dalam bentuk tiga dimensi. Hidrogel carboxymethyl cellulose (CMC) berangkai silang asid sitrik telah menarik perhatian kerana kebiodegradasiannya, kestabilan mekanikal yang tinggi dan kemampuan menyerap air yang baik. Dalam kajian ini, hidrogel CMC berangkai silang asid sitrik disediakan menggunakan kaedah penyediaan yang berbeza untuk meningkatkan sifat fizikal dan mekanikalnya. Hydrogel CMC berangkai silang asid sitrik dibuat dengan mengubah kepekatan CMC pada 2, 3, dan 4 w/v % dan mengubah kepekatan asid sitrik pada 10, 15, dan 20 mol/mol %. Keadaan pengadukan campuran hydrogel ditetapkan dalam keadaan bilik dan keadaan pemanasan (50 - 55 °C); sementara keadaan pengeringan campuran hidrogel ditetapkan dalam pengeringan di peti sejuk (4 °C) dan pengeringan di ketuhar (60 °C). Analisis SEM dan BET mendedahkan bahawa hidrogel tidak berpori dan penyerapan air berlaku melalui interaksi ionik antara molekul air dan CMC. Terbukti kepekatan CMC dan asid sitrik yang lebih tinggi akan meningkatkan ketumpatan silang. Ketumpatan penghubung silang yang tinggi mengurangkan nisbah bengkak dan nisbah penurunan. Komposisi hidrogel 4 w/v % CMC 20 m/m % asid sitrik yang disediakan dengan mengaduk pada keadaan pemanasan dan dikeringkan dengan ketuhar adalah hidrogel yang paling stabil dengan nisbah pembengkakan 1879.89 % dan nisbah degradasi yang serendah 12.07 %.

# **PREPARATION AND CHARACTERIZATION OF CITRIC ACID CROSS-LINKED CARBOXYMETHYLCELLULOSE HYDROGEL**

## **ABSTRACT**

Hydrogel is a network of water-containing flexible polymer chains physically or chemically cross-linked in three-dimensional form. Citric acid cross-linked carboxymethyl cellulose (CMC) hydrogels have attracted attention for their biodegradability, high mechanical stability, and good water-absorbing ability. In this study, citric acid cross-linked CMC hydrogel was prepared using different preparation methods to enhance its physical and mechanical properties. The citric acid cross-linked CMC hydrogel was prepared by varying the concentration of CMC at 2, 3, and 4 w/v % and varying the concentration of citric acid at 10, 15, and 20 mol/mol %. The stirring condition of hydrogel was varied at room temperature and heating (50 - 55 °C); while the drying condition of hydrogel was varied at fridge-dried (4 °C) and oven-dried (60 °C). The SEM and BET analysis revealed that the hydrogel is non-porous and water absorption occurred via hydrogen bonding between water and CMC molecules. It is proven that a higher concentration of CMC and citric acid increased the cross-linking density. High cross-linking density significantly decreased the swelling ratio and degradation ratio. The hydrogel composition of 4 w/v % CMC 20 m/m % citric acid prepared by stirring at a heating condition and oven-dried was the most stable hydrogel with a swelling ratio of 1879.89 % and a low degradation ratio of 12.07 %.

# CHAPTER 1

## INTRODUCTION

### 1.1 Background

Hydrogel is a network of water-containing flexible polymer chains physically or chemically cross-linked in three-dimensional form (Kumar and Erothu, 2016). It is also known as the superabsorbent hydrogel, as its water-absorbing ability can be more than 100 times from its dry weight (Guilherme et al., 2015). Hydrogel is widely applied in hygienic products but owing to its good water-absorbing ability, its application in other fields is gaining attention, such as the drug delivery system, agricultural field, and wastewater treatment (Fekete et al., 2017). The source of hydrogels can be obtained from synthetic or natural polymer.

Synthetic polymer hydrogel is first developed by Wichterle and Lim, which is applied in contact lens production (Wichterle and Lim, 1960). It is also applicable in drug delivery (He et al., 2006), agricultural fields (Ramli et al., 2019), and wastewater treatment such as pollutants removal (Wu et al., 2017; Tang et al., 2010). However, most of the synthetic polymer hydrogels utilize toxic materials and have extensive downstream processing steps (Lee and Mooney, 2001). With the drawbacks stated and the low biodegradability of synthetic polymer hydrogel, the focus is to lead back to natural polymer hydrogel.

Natural polymer hydrogel is relatively eco-friendly, non-toxic, and biodegradable compared to synthetic polymer hydrogel (Guilherme et al., 2015). Natural polymers have been used widely to replace or combined with conventional synthetic polymers such as acrylic polymers to produce hydrogel (Khan and Lo, 2016). In this study, a cellulose derivative, carboxymethyl cellulose (CMC) is proposed as the raw material for hydrogel synthesis. From the perspective of availability in Malaysia,

CMC can be obtained by the etherification of cellulose from Malaysian agricultural wastes (Huang et al., 2017b). However, as natural polymer tends to have lower mechanical stability. Chemical cross-linking is required to improve the mechanical strength of CMC (Chan et al., 2020). The cross-linking agent used is citric acid, which is non-toxic and low cost. Based on Demitri et al., (2008), citric acid cross-linking can produce resistant hydrogel with optimum flexibility even at low citric acid concentration during hydrogel synthesis.

Studies on the different preparation methods of citric acid cross-linked CMC hydrogels are still scarce. Hence, in this study, a natural polymer hydrogel derived from CMC with citric acid cross-linker was prepared using different preparation methods. The effect on the mechanical strength and characteristics of the hydrogels based on their different preparation method were studied.

## **1.2 Problem Statement**

Among the natural polymers, CMC is a promising material for hydrogel owing to its advantage in water adsorption and biodegradability. CMC-based hydrogel is reported to possess similar swelling properties as acrylate-originated products (Durpekova et al., 2020).

As natural polymer hydrogels such as CMC hydrogels typically have less mechanical stability compared to synthetic polymer hydrogels, therefore, a cross-linking technique has to be performed to enhance their mechanical properties (Bao et al., 2019). To maintain the effective absorbing properties of hydrogels while enhancing their mechanical strength, chemical cross-linking was practiced. However, the conventional chemical cross-linkers used such as formaldehyde-based compounds,

sodium polyacrylates, divinyl sulphone (DVS), and carbodiimide are mainly toxic or expensive (Demitri et al., 2008).

A cross-linker that can overcome the toxicity and high-cost problem is citric acid. The presence of citric acid as a cross-linker in natural polymer hydrogels can efficiently enhance their mechanical strength (Bao et al., 2019; Durpekova et al., 2020). To date, the available works on citric acid cross-linked CMC hydrogels focused on the characterization, gel fraction, and swelling properties (Demitri et al., 2008; Erceg et al., 2020; Takigami et al., 2012; Takigami et al., 2009). Comparison between the preparation method of citric acid cross-linked CMC hydrogels has not been reported. Furthermore, CMC is mostly applied as copolymers with starch or other polysaccharides (Fekete et al., 2017; Mali et al., 2018).

Hence, this study focused on the preparation method of pure CMC hydrogels with citric acid as cross-linkers. The effect on the mechanical strength and characteristics of the hydrogels based on their different preparation method was also studied in-depth.

### **1.3 Objectives**

- i. To synthesize the citric acid cross-linked CMC hydrogel using different preparation methods.
- ii. To characterize the physical and the mechanical properties of the citric acid cross-linked CMC hydrogel.

### **1.4 Thesis Organization**

The study mainly focuses on the effect of the preparation method on the strength and characteristics of citric acid cross-linked CMC hydrogel.



Chapter 1 (Introduction) shows a brief overview about the research study on natural polymer hydrogels with non-toxic cross-linking agents. Problem statement is highlighted by identifying the research gaps, whereby none of the literature works reported different preparation methods to synthesize the above-mentioned hydrogels. The objectives and the thesis organization are included in the last section of this chapter.

Chapter 2 (Literature Review) summarizes the overall review regarding the synthesis of citric acid cross-linked CMC hydrogel. The materials, cross-linking agents and methods as well as the potential applications of the natural polymer hydrogel are discussed in detail in this chapter.

Chapter 3 (Methodology) demonstrates a list of materials and equipment required for the experimental studies. The experimental procedures includes different preparation and drying conditions of the citric acid cross-linked CMC hydrogels. The related procedures regarding swelling ratio and degradation ratio measurements are listed with the related equations. The analytical techniques regarding the characterization of the hydrogel are explained.

Chapter 4 (Results and Discussion) presents a complete discussion based on the research results. The effect of different preparation methods on the physical, mechanical properties and characterizations of the synthesized hydrogels are discussed.

Chapter 5 (Conclusions and Recommendation) displays the conclusions drawn based on the results and discussions of this research work. The recommendations are also outlined for the future research regarding this study.

**CHAPTER 2**  
**LITERATURE REVIEW**

**2.1 Hydrogel**

A hydrogel is a flexible three-dimensional (3D) network with specific functional groups (Khan and Lo, 2016). It can be classified based on source and type of cross-linking as shown in Figure 2.1 (Bao et al., 2019; Samaddar et al., 2019).

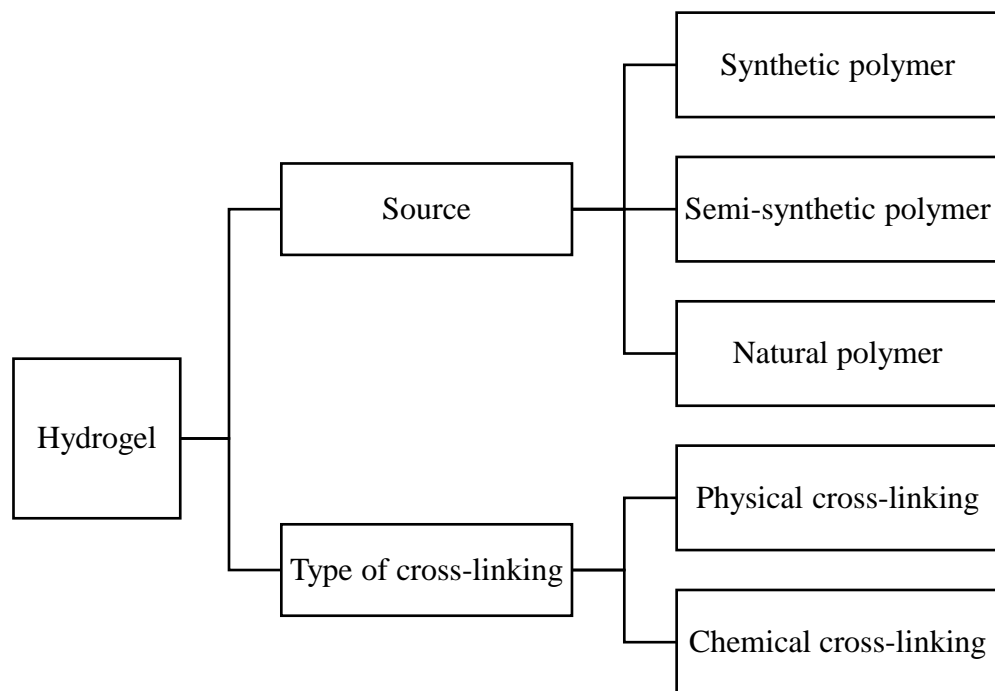


Figure 2.1 Classification of hydrogels

## **2.2 Source of Hydrogel**

### **2.2.1 Synthetic and Semi-Synthetic Polymer Hydrogel**

Synthetic polymer hydrogel is synthesized using the chemical polymerization of synthetic monomers. Some examples of synthetic polymer hydrogel are based on polyvinyl alcohol (PVA), acrylic acid, and polylactic acid (PLA). The synthetic polymer hydrogel typically offers precise controllability of the composition required in preparing the desired hydrogel properties for its specific application (Gupta and Singhal, 2015).

A synthetic polymer hydrogel was prepared using PVA and aluminum oxide ( $\text{Al}_2\text{O}_3$ ) at a slight heating condition (Li et al., 2020). The PVA network was cross-linked by supporting activated alumina to improve the mechanical strength of the synthetic polymer hydrogel. The porous hydrogel synthesized has a uniform pore size and a loose PVA structure, which facilitates the diffusion of water molecules and pollutants into the channels of the hydrogel. The PVA/ $\text{Al}_2\text{O}_3$  hydrogel can be applied as a potential adsorbent for phosphorus removal from wastewater. The spent hydrogel after adsorption can be regenerated, indicating good mechanical stability and recycling properties.

However, synthetic polymer hydrogels have low biodegradability. Owing to this property, they can potentially pollute the soil (Durpekova et al., 2020). The low biodegradability also limits the applications for drug delivery whereby these synthetic polymer hydrogels will impose toxicity and irritation to human body (Kim et al., 2014). Other than the low biodegradable properties, toxic wastes can be released from the synthesis environment of synthetic polymer hydrogel. Synthetic polymers like polyethylene glycol (PEG) are petroleum-based components and produced from non-renewable resources. For example, polyethylene glycol is commercially produced from

ethylene oxide (Bailey, 2012). The waste from the production and possibly the contaminant in the end-product includes the unreacted ethylene oxide. Based on the National Toxicology Program, ethylene oxide is a human carcinogen and could increase risk of cancer during exposure (Marsh et al., 2019). PEG compounds also highlight the evidence of genotoxicity. It can act as penetration enhancer (Zhi et al., 2009), which can increase the human skin's permeability and more susceptible to reject harmful substances during exposure. Hence, the usage of synthetic polymer has various health and sustainability issues.

Semi-synthetic polymer hydrogel is the hydrogel that combines the natural polymer with chemical components in its 3D structure (Gupta and Singhal, 2015). Such polymer hydrogel includes hydrogel synthesized using poly(N-isopropyl acrylamide) (PNIPAM) and sodium alginate (Luo et al., 2019). The natural polymer used was sodium alginate and the synthetic polymer used is PNIPAM. The hydrogel was physically cross-linked using zirconium ions. The hydrogel was prepared at room temperature (25 °C) with 12 hours of stirring to obtain a homogenized mixture for hydrogel synthesis. The highest swelling ratio achieved by altering the polymeric compositions of the hydrogel was 6.47 g/g.

There are various synthetic and semi-synthetic polymer hydrogels reported previously. However, the semi-synthetic polymer hydrogel is still not environmentally friendly because the synthetic portion can release toxic substances during its production. For example, the PNIPAM is typically produced from methanol, which is a derivative from conventional fossil-fuel sources that are non-renewable (Ghasemzadeh et al., 2018; Biswas et al., 2012). The cytotoxicity of PNIPAM hydrogel was caused by the presence of impurities such as its leftover monomer during its production (Yogev et al., 2019). The monomer of PNIPAM is N-isopropylacrylamide,

which is a toxic substance (Cooperstein and Canavan, 2013). Based on the Globally Harmonized System classification, the exposure to N-isopropylacrylamide will induce serious eye damage or irritation (Category 1) and it has acute toxicity (Category 4) if exposed to human (Sigma-Aldrich, 2021). Table 2.1 shows a list of synthetic and semi-synthetic hydrogels with their characteristics and potential applications.

Table 2.1 Synthetic and semi-synthetic polymer hydrogels

Types of polymer	Hydrogel's material	Characteristics	Potential applications	References
Synthetic polymer	Poly(methacrylic acid)/poly(hydroxyethyl methacrylate)	<ul style="list-style-type: none"> <li>• Porous with three functional layers, swelling, non-swelling and mucoadhesive layer for drug entrapment</li> <li>• Highest swelling ratio of 20 g/g and swollen at all pH condition in buffer solution consisted of citric acid and sodium phosphate</li> <li>• Self-folding ability when swollen improved mucoadhesion and enhanced uni-directional drug delivery</li> </ul>	Drug delivery	(He et al., 2006)
	Poly(acrylic acid)/NPK	<ul style="list-style-type: none"> <li>• Porous with loose structure under SEM analysis</li> <li>• Swelling ratio of 165 g/g in distilled water</li> <li>• Remained stable when buried in soil for 2 weeks and completely degraded after 12 weeks</li> <li>• Porous and loose structure facilitates absorption and controlled release of water and NPK fertilizer</li> </ul>	Slow release fertilizer	(Ramli et al., 2019)

	Poly(aspartic acid)/ poly(acrylic acid)	<ul style="list-style-type: none"> <li>• Porous with BET surface area of 62.73 m<sup>2</sup>/g</li> <li>• Highest swelling ratio of 73.3 g/g in deionized water</li> <li>• Regeneration ability up to six cycles of adsorption and desorption</li> </ul>	Methylene blue and neural red dye removal	(Jv et al., 2019)
Semi- synthetic polymer	CMC/poly(lactic acid-co- itaconic acid)	<ul style="list-style-type: none"> <li>• Porous hydrogel that decomposed at 450 °C</li> <li>• Swelling ratio of 332 % in distilled water at 60 °C</li> <li>• Maximum amoxicillin drug loading of 48 % in distilled water</li> <li>• Maximum amoxicillin drug release at pH 2.2 (immersed in simulated gastric fluid)</li> </ul>	Drug delivery	(Sood et al., 2017)
	CMC/acrylic acid	<ul style="list-style-type: none"> <li>• Highest swelling ratio of 16500 % in distilled water at 27 °C</li> <li>• Degraded 40 % after 4 weeks buried in soil</li> <li>• High water retention ability increases germination percentage and germination energy of plant when buried in soil</li> </ul>	Water reservoir	(Sutradhar et al., 2015)

	<p>Polyvinyl alcohol/chitosan/poly (acrylic acid-co-acrylamide)</p>	<ul style="list-style-type: none"> <li>• Porous trilayer coated hydrogel that degraded 42.83 % at 590 °C</li> <li>• Swelling ratio of 233 g/g in deionized water at room temperature</li> <li>• Released 84 % of nitrogen, 63 % of phosphorus, and 36 % of potassium after 30 days in deionized water</li> </ul>	<p>Controlled release fertilizer</p>	<p>(Noppaku ndilograt et al., 2015)</p>
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### 2.2.2 Natural Polymer Hydrogel

Natural polymer hydrogels have a higher biodegradability and lower cost compared to synthetic polymer hydrogel (Khan and Lo, 2016; Guilherme et al., 2015). The typical preparation of natural polymer hydrogels is at room temperature which is energy-saving (Siwek et al., 2019; Guilherme et al., 2015). Some examples of natural polymer hydrogel are goethite, chitosan, and CMC.

A natural polymer hydrogel, alginate/goethite composite hydrogel was synthesized in Siwek et al. (2019) using the encapsulation technique. The sodium alginate was cross-linked with calcium chloride to enhance the mechanical stability of the hydrogel. The synthesis of this hydrogel was done at room temperature. The composite hydrogel was tested for its mechanical stability by using a strength-tester. It was found out that the composite hydrogel can retain its mechanical strength until 28 days in deionized water. However, despite the good mechanical stability, hydrogel synthesized from the encapsulation technique has reduced flexibility (Amsden and Turner, 1999).

Dai et al. (2020) reported on another natural polymer, chitosan hydrogel which was prepared with self-crosslinked cationic guar gum (CGG). The cross-linking agent was sodium periodate ( $\text{NaIO}_4$ ). The preparation of hydrogel was done at room temperature with a stirring time of 12 hours for homogenization. The amount of  $\text{NaIO}_4$  added for cross-linking is 8 w/w % to the amount of chitosan used. The swelling ratio of the hydrogel can reach 3000 % after immersing in water for 24 hours. The hydrogel has a pore area of  $4.34 \pm 0.48 \text{ m}^2/\text{g}$  when inspected with mercury intrusion porosimetry, indicating that it has a porous structure. However, hydrogel synthesized from chitosan has a relatively short lifespan when exposed to microorganisms present in water bodies and has an increased price over the years (Meister et al., 2019).

Palantöken et al. (2020) reported that hydrogel can be synthesized from cellulose with glycine as a cross-linker. The hydrogel mixture was prepared by stirring at ice bath (0 – 4 °C) for 2 hours and dried at room temperature overnight. The hydrogel cross-linked with 30 vol % of glycine can reach a swelling ratio of 700 %. The hydrogel can withstand temperature up to 280 °C. However, the swelling ratio is still considered low using cellulose as the main composition of hydrogel. Hence, alternatives for a more hydrophilic natural polymer are sought to produce a hydrogel which possesses high swelling ratio and good mechanical strength. Table 2.2 shows a list of natural polymer hydrogels. Examples of natural polymer hydrogels from works of literature are shown with their respective cross-linking method, special features and characteristics with their corresponding potential applications.

Table 2.2 Natural polymer hydrogels

Materials	Cross-linking methods	Characteristics	Potential applications	References
Carboxylated chitosan/hydrochloric acid	Chemical cross-linking	<ul style="list-style-type: none"> <li>• Transparent and flexible film</li> <li>• Degraded at 250 – 500 °C</li> <li>• Various hydrophilic groups enhanced electrolyte absorption capacity and ionic conductivity</li> <li>• High electrolyte absorption of 742 %</li> <li>• High ionic conductivity of <math>8.69 \times 10^{-2}</math> S/cm</li> <li>• Both performance indicators of the synthesized film were higher than the commonly reported gel polymer electrolyte</li> </ul>	Energy storage as supercapacitors	(Yang et al., 2019)
Carboxymethyl chitosan/cellulose nanocrystals	Chemical cross-linking	<ul style="list-style-type: none"> <li>• Self-healing and strong hydrogel cross-linked with cellulose nanocrystals</li> </ul>	Burn wound healing and support matrix for cell growth	(Huang et al., 2018)

		<ul style="list-style-type: none"> <li>• Self-healing efficiency of 5 minutes and gelation time of 2 minutes</li> <li>• Swelling ratio of 350 % immersed in distilled water</li> <li>• Cell viability of 97.3 % in 7 days</li> <li>• Self-healing properties reduced time and pain to heal burn wound and prevented scar formation</li> <li>• Can be removed on-demand by dissolving hydrogel with amino acid solution</li> </ul>		
Chitosan/halloysite nanotubes	Heat treatment in ethanol solution	<ul style="list-style-type: none"> <li>• Porous hydrogel with pore size of 100–500 <math>\mu\text{m}</math></li> <li>• Remained stable up to 7 compression cycles for 0.71 MPa</li> <li>• Loading efficiency of 45.7 % for chemotherapy agent, doxorubicin drug</li> <li>• Increasing content of halloysite nanotubes in hydrogel will decrease pore size, swelling ratio but increase drug loading efficiency and stability</li> </ul>	Drug delivery	(Huang et al., 2017a)

CMC/dopamine	Enzymatic cross-linking	<ul style="list-style-type: none"> <li>• Porous hydrogel that degraded 40 – 60 % after 1 week in cellulase digestion</li> <li>• Highest swelling ratio at 49 g/g in buffer solution (pH 7.4)</li> <li>• Cell viability up to 89 % using mouse fibroblast cells</li> <li>• Usage of dopamine for cross-linking increased tissue adhesion strength of 2 – 6 folds stronger than the commercialized fibrin glue</li> <li>• Usage of enzymatic cross-linking provided adjustable gelation time, mechanical stability and biocompatibility</li> </ul>	Tissue adhesive	(Zhong et al., 2019)
CMC/aluminium ion/sodium n-dodecyl sulfate	Physical cross-linking with surfactant	<ul style="list-style-type: none"> <li>• Porous hydrogel beads formed with specific dosage of ionic surfactant</li> <li>• Swelling ratio of 218 % in pH 7</li> <li>• Methylene blue removal of 82 mg/g determined by Langmuir isotherm and pseudo-second-order kinetics</li> </ul>	Methylene blue dye removal	(Benhalim a et al., 2017)

		<ul style="list-style-type: none"> <li>• Can be regenerated and exhibited higher adsorption efficiency after regeneration</li> <li>• Presence of undesorbed dye exposed more internal active sites for more dye absorption in the next cycle</li> <li>• Maximum adsorption of 350 mg/g of methylene blue after 3 cycles of adsorption-desorption</li> </ul>		
CMC/potato starch/aluminum sulfate octadecahydrate	Chemical cross-linking	<ul style="list-style-type: none"> <li>• Optimum cross-linking agent concentration at 2.3 % to prevent over or under cross-linked hydrogel</li> <li>• Highest swelling ratio of 73 g/g in distilled water</li> <li>• Decreasing the amount of potato starch yielded higher radish plant growth</li> </ul>	Water reservoir in soil amendment	(Nnadi and Brave, 2011)

### 2.2.3 Carboxymethyl Cellulose Hydrogel

Carboxymethyl cellulose (CMC) hydrogel is a type of natural polymer hydrogel which is soluble in water. It is derived from cellulose via surface modification of cellulose with etherification (Chen et al., 2018). Figure 2.2 shows the chemical structure of CMC. The functional group of CMC hydrogel is a carboxylic group ( $\text{COO}^-$ ) which makes it an anionic hydrogel. The good water-absorbing ability of the CMC hydrogel is owing to the hydrophilic nature of CMC where water molecules can easily penetrate the hydrogel matrix by forming hydrogen bonding with the hydroxyl or carboxylic group of CMC molecules (Zonatto et al., 2017; Samaddar et al., 2019; Demitri et al., 2008).

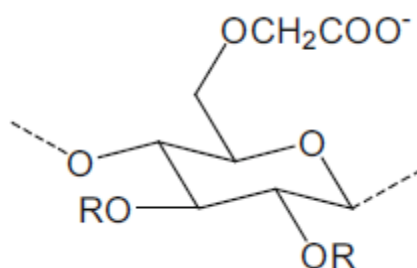


Figure 2.2 Chemical structure of CMC (Chen et al., 2018)

A natural polymer hydrogel can be synthesized using a combination of CMC and starch (Fekete et al., 2017). The prepared hydrogel mixture was stored for 24 hours after stirring for homogeneity. The cross-linking was done using gamma irradiation ( $^{60}\text{Co}$   $\gamma$ -source) to achieve gel fraction without any usage of cross-linking agents. The addition of more starch promotes higher cross-linking density while the addition of more CMC promotes a higher swelling ratio. The sample synthesized with 30 % of starch content and 20 kGy absorbed dose of  $^{60}\text{Co}$   $\gamma$ -source was the optimized sample. The sample has strong mechanical stability with low degradation and a high swelling degree of 350  $\text{g}_{\text{water}}/\text{g}_{\text{gel}}$ . Although the gamma irradiation cross-linking technique does

not require the usage of cross-linking agent, it can cause a certain degree of degradation to the synthesized hydrogel (Fekete et al., 2017).

Demitri et al. (2008) introduced a novel superabsorbent hydrogel synthesized from CMC and hydroxyethyl cellulose (HEC). The mixture composition of CMC to HEC was 3 to 1 weight fraction with low citric acid concentration for cross-linking reaction. The mixture was stirred at room temperature for 24 hours to homogenize it, then 30 °C for 24 hours to remove water and 80 °C for 24 hours to enable cross-linking reaction. The optimal swelling degree obtained was 900 % with 3.75 wt % of citric acid. The hydrogel sample experienced complete degradation at a heating condition of 160 °C. The degradation study by immersing the hydrogel sample in water is not conducted for this work of literature.

Durpekova et al. (2020) also introduced a hydrogel synthesized from similar polymers which are CMC and HEC. The mixture composition of CMC to HEC used was similar to the work reported in Demitri et al. (2008). The stirring of the hydrogel mixture was also carried out for 12 to 24 hours at room temperature. However, the drying and cross-linking temperature of the hydrogel was combined and simplified to 60 °C for 24 hours. The swelling ratio can exceed 1600 %, which was similar to the commercialized synthetic superabsorbent polymer (SAP) hydrogel made from polyarylate. The degradation temperature of the hydrogel was reported at 300 °C to 320 °C. Similarly, the degradation study by immersing the hydrogel sample in water is not conducted for this work of literature.

### **2.2.3.1 Applications of CMC Hydrogels**

CMC-based hydrogels can be applied in various applications including colorimetric sensor for pH an glucose, cell culture, dye removal from wastewater, and drug delivery.



By direct depositing CMC into bacteria cellulose (BC), a hydrogel with loosely packed fibrous network was synthesized (Siripongpreda et al., 2021). Due to the high water absorption ability (a swelling ratio of more than 3000 %), the hydrogel displayed good absorption of the pH sensing elements (indicators). The presence of loose network enabled the pH buffer to penetrate into the hydrogel matrix and perform interactions with the respective pH indicators. The linear detection range for glucose using the synthesized hydrogel was wider with a lower limit of detection and a higher stability. The hydrogel can retain 86 % of the initial enzymatic activity response that produces glucose in 7 days. The enzymatic activity is not retained for CMC hydrogel cross-linked with acidic compounds such as citric acid and aluminium chloride as the acidic nature can denature the enzymes involved for glucose production.

CMC and polyaniline were cross-linked with N,N'-methylenebisacrylamide and acrylic acid to produce a macroporous hydrogel for cationic and anionic dye removal (Bagheri et al., 2021). The hydrogel has antimicrobial properties due to the addition of polyaniline. The cationic dyes used were methylene blue (MB) and rhodamine-B (RhB) while the anionic dye used was methyl orange (MO). Due to the cross-linked structure, the hydrogel displayed 47 % of degradation after 110 days buried in the soil. Owing to the amine and imine groups of polyaniline in hydrogel that exhibited good interaction with the pollutants in the wastewater, the highest achieved removal efficiency for MB, RhB, and MO were 97.5 %, 96 % and 74% respectively. All three dyes were best described by pseudo-second-order model. MB and RhB best fit in Langmuir isotherm while MO best fit in Freundlich isotherm.

A hydrogel was synthesized by incorporating magnetic iron oxide nanoparticles (MIONs) into CMC without using any chemical cross-linking agent (Kurdtabar et al., 2018). The highest swelling ratio reported was 428 g/g in distilled water. The presence

of hydroxyl and carboxyl groups in CMC hydrogel promotes hydrogen bonding with the doxorubicin drug used for the drug release studies. The amount of drug release increased with the pH and optimum at pH 7 (90 % of drug released). This could be due to the water absorbing ability was also optimum at this condition. The hydrogel did not show any cytotoxicity effects towards the human umbilical vein endothelial cells, showing good biocompatibility to be a potential tissue filler.

### **2.3 Types of Cross-Linking of Hydrogel**

Cross-linking is a technique that improves the mechanical stability and swelling extent of polymer hydrogels (Khan and Lo, 2016). Natural polymer hydrogels are initially weak in mechanical stability. Hence, cross-linking is required to strengthen the 3D hydrogel network structure (Bao et al., 2019). The two main types of cross-linking techniques are physical cross-linking and chemical cross-linking.

#### **2.3.1 Physical Cross-Linking**

The physical cross-linking technique is done via non-covalent interactions (Wang et al., 2019). This cross-linking technique can increase the porosity of CMC hydrogel (Zhang et al., 2018b). The main physical cross-linking technique is ionic cross-linking (Liang et al., 2019). This can be due to the advantage that ionic cross-linking can happen at a mild condition with room temperature and physiological pH (Maitra and Shukla, 2014).

Ionic cross-linking using iron (Fe) is a widely used physical cross-linking technique to strengthen the mechanical structure of natural polymer hydrogels due to the strong buffering ability exhibit by the trivalent iron ions (Zhang et al., 2018a). A CMC hydrogel physically cross-linked with Fe ions (CMC-Fe hydrogel) was synthesized using 1.5% of CMC concentration (Ure and Mutus, 2021). The drying

condition was at 40 °C for 24 hours in a vacuum oven. The application of this hydrogel was on the removal of inorganic phosphate. However, the hydrogel sample was prone to degrade due to the leaching of the Fe cross-linking agent at an acidic or an alkaline condition.

The zirconium ions (Zr (IV)) can also be used to ionically cross-link the CMC hydrogel to increase its porosity and total surface area (Wang and Wei, 2021). The CMC/carboxymethyl chitosan (CMCS) hydrogel was cross-linked with Zr (IV), whereas polyethylene glycol (PEG) was used as the pore-forming agent to produce a macroporous structure. The formation of pores was facilitated to promote the adsorption of pollutants from the wastewater as the hydrogel will be applied in pollutants removal. The Zr (IV) ions were obtained from analytical graded zirconium oxychloride octahydrate ( $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ ). The stirring of the hydrogel mixture was done at room temperature. The hydrogel was eluted in water for 48 hours to remove the excess PEG after the pore-forming stage. However,  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$  that was used to provide Zr (IV) for ionic cross-linking is corrosive (LobaChemie Pvt. Ltd., 2016).

The leaching of Fe cross-linker shows the CMC hydrogel cross-linked physically tends to have unstable structure, most probably due to the non-covalent bonding. Moreover, the quality control of hydrogels produced is relatively difficult compared to chemical cross-linking techniques due to the unstable time of gelation and different pore size of the hydrogel matrix structure (Samaddar et al., 2019). Hence, the drawbacks of physical cross-linking lead to the development of the chemical cross-linking technique.

CMC hydrogel was prepared by mixing it with PVA and reinforced with graphene oxide and bentonite to improve the performance of the hydrogel (Dai et al., 2018). The hydrogel was stirred at 95 °C for 3 hours. The highest swelling ratio reached

when immersed in distilled water was 35.23 %. The synthesized hydrogel degraded above 200 °C, showing good mechanical strength under high temperature. The hydrogel has a high reusability as it was used to conduct adsorption-desorption of methylene blue (MB) for four cycles.

### **2.3.2 Chemical Cross-Linking**

Chemical cross-linking is more preferable due to its relatively stable covalent interactions formed within the long-chain polymer network in a hydrogel. However, cross-linkers used in chemically cross-linked hydrogels usually are toxic, such as glutaraldehyde and formaldehyde (Tenório et al., 2020). This causes the hydrogel produced to be not eco-friendly and non-biodegradable. A few examples of toxic and non-toxic cross-linking agents have been reviewed for their water-absorbing properties and application in removing pollutants from wastewater.

A CMC hydrogel was chemically cross-linked with magnetite nanoparticles using cross-linker 3-aminopropyltrimethoxysilane (APTMS) (Uva et al., 2017). The swelling ratio test was done in deionized (DI) water and it can reach 7490 %. The swelling ratio remained constant after 2 hours of immersion in DI water. The degradation of the hydrogel was not studied in this work. Although the reported swelling ratio was high, the usage of toxic cross-linker can cause secondary pollution to the environment where the spent hydrogel can be non-biodegradable. The disposal of the toxic content in the cross-linked hydrogels can create additional problems for the environment.

Another chemical cross-linker to enhance the mechanical stability of CMC hydrogel was ethylenediamine (EDA) (Velepini et al., 2017). The prepared mixture was left at 45 °C for 24 hours for cross-linking reaction to happen. The hydrogel sample was left in a vacuum oven for 50 °C for drying purposes. The hydrogel was used to

adsorb chromium ions (Cr (VI)) from wastewater. The regeneration of the hydrogel was feasible using 0.1 M of sodium hydroxide. Although EDA as a cross-linker can maintain the structure of hydrogel even after few cycles of regeneration, its toxicity and non-degradability can be the drawbacks of the cross-linked hydrogel. The drawbacks of the toxic cross-linkers for chemically cross-linked hydrogels open the opportunities for the application of non-toxic cross-linkers which are much more environmentally friendly.

### **2.3.3 Citric Acid Cross-Linked CMC Hydrogel**

A common non-toxic and low-cost natural cross-linker used to chemically cross-link cellulose-derived hydrogel is citric acid (Dharmalingam and Anandalakshmi, 2019). Studies have been presented that citric acid is capable to cross-link cellulose-derived hydrogels in agricultural and drug delivery applications (Dharmalingam and Anandalakshmi, 2019; Erceg et al., 2020). This is because citric acid can perform a similar cross-linking effect like the high-performance toxic cross-linkers. Compared with other polycarboxylic acids, citric acid is relatively cheap and has a milder treatment condition for cross-linking (Fekete and Borsa, 2018).

The cross-linking mechanism between citric acid and CMC is demonstrated in Figure 2.3. Under dry and heating condition, citric acid will expose its carboxyl (COOH) group to form citric acid anhydride for cross-linking reaction. The hydroxyl (OH) group of the CMC will form a covalent ester bond with the anhydride group of the citric acid (Capanema et al., 2018; Demitri et al., 2008). As one molecule of the citric acid has three carboxylic groups, three ester bondings can be formed by reacting the citric acid with CMC. The cross-linking reaction (specifically known as the esterification (Mali et al., 2018)) can effectively strengthen the rigidity of the citric acid cross-linked CMC hydrogel network.