BIO-RENEWABLE HYDROGEL SORBENTS FOR REMOVAL OF HEAVY METALS

BIO-RENEWABLE HYDROGEL SORBENTS FOR REMOVAL OF HEAVY METALS FROM WATER

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Master of Science Degree

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Lay Abstract

Heavy metal pollution persists in the environment and can lead to bioaccumulation and toxicity. This problem severely impacts resource-limited areas where drinking water is already scarce. Various filtration systems for heavy metal removal have been developed, with sorption being the most cost-effective and simplest to use. Sorption is the accumulation of substances both at a surface and inside the bulk material, which is called a sorbent. Many commercial sorbents are powders that lead to inefficient sorbent recovery and recyclability, as well as secondary pollution. Therefore, in this work, we have explored the use of cellulose — the most abundant biopolymer on Earth — to develop hydrogels for removal of heavy metals from water. Hydrogels are three-dimensional, crosslinked, hydrophilic (or water-loving) polymeric networks. A key feature of our hydrogels is their bio-renewable, non-toxic, and biodegradable composition, thus addressing UN Sustainable Development Goal # 6 (clean water and sanitation for all) while implementing the principles of green chemistry. We used two types of modified cellulose polymers; one provides structural support, and the other binds metal ions, resulting in a maximum binding capacity of 102 mg of copper per gram of dry hydrogel. While these polymers offer flexibility to the hydrogel structure, we need mechanically strong hydrogels to strive towards their future use in the wastewater treatment/metal recovery industry. Therefore, we also studied the incorporation of (modified)-cellulose nanocrystals (CNCs) as rigid green nano-additives to obtain robust nanocomposite cellulose hydrogels. These experienced less extreme swelling and shrinking behavior while maintaining copper binding capacity comparable to our CNC-free hydrogel system. Overall, the findings of our work further contribute to the development and understanding of functional cellulose-based (nanocomposite) hydrogels which have the potential to become inexpensive bio-renewable technologies to clean industrial wastewaters of heavy metals.

Abstract

Water is essential for life, yet water scarcity from heavy metal pollution is a growing problem severely affecting resource-limited areas where drinking water is already lacking. Sorption is the simplest and most economically feasible technique for heavy metal removal; however, many commercial sorbents are powders which have safety concerns, present processing and handling difficulty with low removal efficiency, and potential secondary pollution. To overcome these issues, the goal of this work was to develop an inexpensive, renewable, and biodegradable hydrogel able to efficiently bind heavy metals while practicing the principles of green chemistry. Therefore, we used cellulose, the most abundant and easily degradable biopolymer on Earth. Cellulose derivatives — hydroxyethyl cellulose (HEC), which provides structural support, and carboxymethyl cellulose (CMC), which efficiently binds heavy metals with its wealth of carboxylate groups — were modified with aromatic aldehydes (aa-HEC and aa-CMC). These functionalized cellulose derivatives covalently crosslinked with were an ethylenediaminetetraacetic acid (EDTA)-based crosslinker modified with four hydrazide groups (4h-EDTA) to construct hydrazone crosslinked hydrogels. In Chapter 2, rheology, a method to quantify mechanical strength, was used to optimize the aa-HEC/aa-CMC/4h-EDTA cellulose hydrogels for their crosslinking ratio and composition, determined to be 1:2 aldehyde:hydrazide (a:h) and 2 wt% 1:3 aa-HEC/aa-CMC (1:3 H:C), respectively. This optimal 1:3 H:C hydrogel exhibited a storage modulus (G') of 200 Pa and a maximum sorption capacity of 102 mg/g for Cu²⁺, comparable to current bio-based sorbents. The findings from Chapter 2 provided us with a better understanding of our cellulose-based hydrogels and highlighted the need to enhance their mechanical strength. Thus, in Chapter 3 we explored (modified)-cellulose nanocrystals (CNCs) as rigid green nano-additives in place of a portion of the flexible cellulose derivatives to improve the

hydrogel's mechanical integrity. Specifically, we studied the incorporation of (modified)-CNCs at a 2 wt% 1:1:1 aa-HEC/aa-CMC/(modified)-CNC ratio using our 4h-EDTA crosslinker to form hydrazone bonds at the 1:2 a:h crosslinking ratio. The control condition used native CNCs, and the modified-CNCs included aromatic aldehyde modified-CNCs (aa-CNCs) and carboxylated CNCs (T-CNCs). All nanocomposite hydrogels (1:1:1 CNC, 1:1:1 aa-CNC, and 1:1:1 T-CNC) showed decreased swelling and greater mechanical strength compared to the 1:3 H:C hydrogel after salt/buffer incubation. Additionally, after incubation in excess Cu²⁺, all hydrogel compositions experienced shrinking which significantly enhanced their mechanical strength --- the 1:1:1 T-CNC gained the most strength (G' of 150 Pa pre-incubation to 3100 Pa post-incubation in Cu²⁺). Furthermore, sorption studies revealed the 1:1:1 T-CNC composition had a binding capacity of 90 mg/g for Cu²⁺, comparable to our 1:3 H:C hydrogel and current bio-based sorbents. Overall, our findings provided us with a blueprint towards using functionalized cellulose derivatives and modified-CNCs to develop mechanically strong nanocomposite cellulose hydrogels. These cellulose-based hydrogels have the potential to serve as safe, sustainable, inexpensive, and easyto-handle alternatives to powdered sorbents for water purification of heavy metals.

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List of Abbreviations

4h-EDTA	Tetrahydrazide-ethylenediaminetetraacetic acid
4h-EDTA_a-CNC	4h-EDTA modified-aldehyde cellulose nanocrystals
aa-CMC	Aromatic aldehyde modified-carboxymethyl cellulose
aa-HEC	Aromatic aldehyde modified-hydroxyethyl cellulose
a:h	aldehyde:hydrazide
a-CNC	Aldehyde modified-cellulose nanocrystals
aa-CNC	Aromatic aldehyde modified-cellulose nanocrystals
As	Arsenic
Cd	Cadmium
CMC	Carboxymethyl cellulose
CNCs	Cellulose nanocrystals
Co	Cobalt
Cr	Chromium
Cu	Copper
DCM	Dichloromethane
DoF	Degree of functionalization
DLS	Dynamic Light Scattering
EDTA	Ethylenediaminetetraacetic acid
G'	Storage modulus
G"	Loss modulus
HEC	Hydroxyethyl cellulose
Hg	Mercury

Mg	Magnesium
M _n	Number-average Molecular Weight
MW	Molecular Weight
NaCl	Sodium chloride
Ni	Nickel
NMR	Nuclear magnetic resonance
PBS	Phosphate buffered saline
PDMS	Polydimethylsiloxane
PEI	Poly(ethyleneimine)
Pb	Lead
RT	Room temperature
T-CNC	Carboxylated CNC
THF	Tetrahydrofuran
UV-vis	Ultraviolet-visible
Zn	Zinc
ZP	Zeta potential

Declaration of Academic Achievement

I declare that the research contribution which follows is original work, that I, Manjot Grewal, have completed and written, with editorial assistance from Dr. Jose M. Moran-Mirabal. The following individuals contributed to experimentation and data analysis for Chapter 2: Dr. Ayodele Fatona and Yue Su assisted with synthesis methods and Julia Ungureanu assisted with rheology measurements. Dr. Jose M. Moran-Mirabal provided research oversight and funding and reviewed all drafts of Chapter 2 to prepare and submit a manuscript for publication to ACS Applied Materials & Interfaces.

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- (2) Merlo, A.; González-Martínez, E.; Saad, K.; Gomez, M.; Grewal, M.; Deering, J.; DiCecco, L.-A.; Hosseinidoust, Z.; N. Sask, K.; M. Moran-Mirabal, J.; et al. Functionalization of 3D Printed Scaffolds Using Polydopamine and Silver Nanoparticles for Bone-Interfacing Applications. *ACS Appl. Bio Mater.* 2023, *6* (3), 1161–1172.
- (3) Halimi, I.; Rodrigues, E. M.; Maurizio, S. L.; Sun, H.-Q. T.; Grewal, M.; Boase, E. M.; Liu, N.; Marin, R.; Hemmer, E. Pick Your Precursor! Tailoring the Size and Crystal Phase of Microwave-Synthesized Sub-10 Nm Upconverting Nanoparticles. *J. Mater. Chem. C* 2019, *7* (48), 15364–15374.

Chapter 1: Introduction & Literature Review 1.1 Introduction

Due to increased industrial activities, electronic waste, agricultural and urban run-off, corrosion of pipes and plumbing systems, natural weathering and more, the rise of heavy metal pollution poses a significant risk to human health and the environment. Heavy metal pollution of our finite freshwater sources is a growing problem worldwide, especially in resource-limited areas where drinking water is already scarce. To address this issue, a variety of water purification technologies. have been developed, with sorption being the most economically viable.^{1,2} Sorption materials such as carbon nanotubes, graphene derivatives, activated carbon, metallic nanoparticles and zeolites have been investigated;³ however, the material costs and toxicity risks of nanomaterial sorbents have prompted the development of economically and environmentally sustainable alternatives.^{4,5} Chapter 1 provides context on major water pollutants and water purification technologies before introducing sorption technology and sorbent materials to address heavy metal pollution. This is followed by a discussion of the need for hydrogels as alternatives to current powdered sorbents. Finally, cellulose is discussed in detail as the biomaterial of choice to undertake the goal of addressing the need for environmentally benign heavy metal binding hydrogel sorbent materials.

The overarching goal of this work was to develop a cost-effective, bio-renewable, and biodegradable hydrogel able to efficiently chelate heavy metals using the principles of green chemistry (7-use of renewable feedstock and 10-design for degradation). To accomplish this, cellulose — the most abundant bio-renewable polymer on Earth was used.⁶ Specifically, cellulose derivatives, hydroxyethyl/carboxymethyl cellulose (HEC/CMC), were modified with aromatic aldehydes (aa-HEC/aa-CMC) and crosslinked with an ethylenediaminetetraacetic acid (EDTA)-based crosslinker modified with four hydrazide groups to make covalently cross-linked hydrogels.

The details of this project are covered in Chapter 2, where the main objectives include: (1) synthesis of the components of the hydrogel; (2) determination of the aldehyde content on the cellulose; (3) optimization of hydrogel crosslinking ratio and composition; (4) evaluation of hydrogel swelling and stability in different environmental conditions; and (5) evaluation of the hydrogel's physicochemical sorption properties. Specifically, for Aim 3, rheological tests were performed to quantitatively determine the ratio of aldehyde:hydrazide that results in robust hydrogels. Then the ratio of aa-HEC/aa-CMC was optimized to obtain the highest content of aa-CMC that maximized the binding capacity of the hydrogels while retaining hydrogels that are robust and easy to handle. For Aim 4, hydrogels were tracked for their swelling behavior over a six-week timeframe upon incubating in solutions with acidic and basic environments (pH 3 and 11), and under different ionic strengths at pH 7 (0-100 mM NaCl). For Aim 5, the physicochemical tests completed include sorption isotherms, sorption kinetics, and a multi-metal sorption study for metal ions (Cu^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} and Mg^{2+}) tested individually or together as a multiplecomponent system. During the physicochemical sorption tests, covered in Chapter 2, critical aspects of the hydrogel design that impact the sorption speed were identified, such as the rapid crosslinking of external layers of the hydrogel upon exposure to heavy metals. These findings provided the framework for improving these hydrogels which were addressed in Chapter 3.

Briefly, the main objective covered in Chapter 3 was enhancing the mechanical strength of the aa-HEC/aa-CMC hydrogels to work towards templating robust hydrogel beads which would have higher surface area and hence faster sorption kinetics, and be able to be used in a column, which is a requirement for scalable real-world use. To progress towards this vision, the primary step was to improve robustness of the hydrogels covered in Chapter 2. This was investigated in Chapter 3 through the incorporation of cellulose nanocrystals (CNCs) as a green additive, including pristine

CNCs, aromatic aldehyde modified CNCs, and carboxylated CNCs into the aa-HEC/aa-CMC hydrogels in equal ratio to the two cellulose polymers (1:1:1). These hydrogel composites were tested for their swelling, mechanical strength and binding capacity for copper. In Chapter 4, the findings covered in Chapter 2 and 3 are summarized alongside next steps to work towards addressing the need for industrially employable robust cellulose hydrogel materials for heavy metal sorption made with the principles of green chemistry.

1.2 Water Quality and its Importance

With the rising pressures of rapid climate change, frequent environmental catastrophes, population growth, industrial and urban development, etc. there has been an alarming increase of toxic pollutants entering the environment.⁷ Water resources worldwide are becoming increasingly strained with two-thirds of the global population experiencing severe water scarcity at least one month per year.8 Even developed nations like the United States have reported one in five Americans are exposed to water contamination.⁹ Freshwater is the most essential natural resource, crucial for human well-being and natural ecosystems. The United Nations Sustainable Development Goal 6 (SDG 6) specifically addresses the importance of water access as a fundamental human right essential for health, sanitation, and economic development.¹⁰ The human body is composed of approximately 50-70% water and requires one litre of water per day for the average adult to stay properly hydrated and maintain a healthy kidney.¹¹ One of the key components of SDG 6 is universal access to safe and affordable drinking water. This is vital as access to clean water is directly linked to health outcomes, with contaminated water being a leading cause of waterborne diseases and mortality among children.¹² Beyond personal consumption, sanitation, cooking and hygiene, water is essential for irrigation, which accounts for up to 70% of global freshwater usage.¹³ Water is also a key asset for energy production and serves

as a universal solvent for water-soluble reagents in the chemical industry. Furthermore, it is used in hardware manufacturing and for cooling the servers that power artificial intelligence computations.¹⁴ Given its widespread applications, maintaining water quality is crucial for both safe consumption and its effective use in different fields.

1.3 Water Pollutants and Sources

Before discussing the various materials and technologies for water purification, it is important to understand the pollutants present in the water matrix. Water pollutants are substances that change the chemical/physical properties of water, deteriorating the quality of water.¹⁵ Based on their composition, pollutants are classified as organic (oils, plastics, dyes, insecticides, pesticides, herbicides, pharmaceuticals, etc.), biological (pathogenic microorganisms), or inorganic (colloidal particles, heavy metal ions, etc.).² At low concentrations, these substances do not cause any harm, but due to many pollutants being non bio-degradable, they persist and accumulate in the ecosystem and in living organisms, causing various health problems depending on their toxicological fingerprint.^{2,16}

Predominant sources of pollution include discharge directly into water streams of hazardous waste from refineries, mines, and the textile, petrochemical and pharmaceutical industries. Water pollutants in treated and recycled water streams include cyanide, phenol, aromatic hydrocarbons, chlorinated hydrocarbons, polycyclic aromatic hydrocarbons, mineral oil, heavy metals and metalloids, and others, of which heavy metals make up the largest normalized composition of 31% (**Figure 1.1**).¹ Heavy metals are defined as metals which have a density above 4-5 g/cm.³ Due to mining and smelting activities, industrial effluents from industries such as electroplating and electronics manufacturing, agricultural run-off (ex. copper-based pesticides and fertilizers leach into water systems), urban run-off (ex. brake pads, roofing materials, etc.), corrosion of pipes and

plumbing systems, and natural sources (ex. weathering and natural disasters), these precious metals are accumulating in terrestrial and aquatic environments, leading to toxicity for both aquatic and terrestrial organisms.² Although several heavy metals serve important physiological functions (ex. copper and iron for oxygen and electron transport; cobalt for cell metabolism; nickel for cell growth; manganese for enzymatic catalysis; selenium for hormone production, etc.) many others (silver, mercury, lead, chromium, cadmium, etc.) can cause detrimental side effects including nerve damage and death.¹⁷ Heavy metals are non-biodegradable and bio-accumulate in living organisms as they consume contaminated water and food.¹⁸ While all living organisms require varying amounts of certain heavy metals for growth, these metals can be toxic when they surpass the maximum allowable concentration (MAC) guideline values (**Table 1.1**).^{18–20} Therefore, it is necessary to purify water of pollutants, with heavy metals being the main pollutant under investigation for this review.



Figure 1.1. (A) Normalized composition of water pollutants in treated and recycled water streams. Reproduced from Bolisetty *et al.*¹ (**B**) Various anthropogenic and natural sources of heavy metal pollution.

Table 1.1. Sources of heavy metal pollution with respective health effects and maximum allowableconcentration (MAC)guidelinevaluesbased on the world health organization (WHO)recommendations. $^{18-20}$

Heavy Metal	Main Sources	Human Health Effects	MAC (mg•L ⁻¹)
Zinc (Zn)	Brass coating, rubber products, some cosmetics and aerosol deodorants.	Anemia, convulsions, stomach cramps, skin irritations, vomiting, and nausea.	3.0
Copper (Cu)	Electronics and cables industry, corroded plumbing systems, and naturally occurring.	Gastrointestinal issues, damage to liver, kidneys, brain, cornea, lungs, immunological system, and hematological system.	2.0
Nickel (Ni)	Stainless steel and nickel alloy production.	Skin, lung, and kidney damage, gastrointestinal distress, and pulmonary fibrosis.	0.07
Chromium (Cr)	Pulp mills and tanneries, steel manufacturing, and naturally occurring.	Diarrhea, nausea, vomiting, allergic dermatitis, damage to lungs, kidneys, liver, brain, pancreas, tastes, and reproductive system.	0.05
Lead (Pb)	Lead-based products (batteries, solder, alloys, cable sheathing pigments, rust inhibitors, ammunition, glazes, and plastic stabilizers), and household plumbing systems.	Reduced neural development, damage to bones, kidneys, lungs, spleen, liver, and the immunological, hematological, cardiovascular, and reproductive systems.	0.01
Arsenic (As)	Electronics and glass production and naturally occurring.	Damage to skin, lungs, kidneys, brain, and the cardiovascular, immunological, metabolic and endocrine systems.	0.010
Mercury (Hg)	Refineries, fossil fuel combustion, electrolytic production of chlorine and caustic soda, runoff from landfills and agriculture, laboratory apparatus, and electrical appliances.	Damage to lungs, kidneys, liver, brain, and the cardiovascular, endocrine, reproductive, nervous and immunological systems.	0.006
Cadmium (Cd)	Metal refineries, batteries, paints, steel and plastic industries, corroded galvanized pipes, and naturally occurring.	Damage to liver, bones, lungs, kidneys, testes, brain, and the immunological and cardiovascular systems.	0.003

1.4 Water Purification Technologies for Heavy Metal Removal

Methods to purify polluted water depend on the water being treated. Generally, the various technologies for treating water can be categorized as physical, chemical or biological treatment methods.² The physical methods include sedimentation, filtration, etc.; the chemical methods include flocculation, coagulation, ion-exchange, chemical precipitation, adsorption, etc.; and the biological methods include bioremediation, aerobic treatment, anerobic treatment, activated sludge, etc. Based on the context of the pollutants present in the water, treatment is often a multistage process including various technologies from all three methods to get from the polluted water to treated water.

1.4.1 Primary and Secondary Water Purification Technologies

To ensure water is treated with high efficiency against all types of pollutants (inorganic, organic and biological), water treatment units combine primary, secondary and tertiary treatment processes (**Figure 1.2**).^{1,21} In the context of heavy metal polluted industrial wastewater, with extensive levels of contamination, with or without oxidized metals, suspended solids and scaling minerals present, it must be pre-treated before undergoing the high quality tertiary treatment process. This prevents higher operational costs and drop of tertiary treatment technology efficiency by avoiding sorbent saturation or membrane fouling.



Figure 1.2. Summary of primary, secondary, and tertiary water treatment technologies to purify wastewater of inorganic, organic and biological pollutants. Adsorption technology is highlighted as the most cost-effective and easy-to-use tertiary treatment technology. Adapted from Kumar *et al.*²¹

Briefly, the primary treatment process can comprise chemical or physical methods, which include microfiltration, screening, sedimentation, centrifugation, flocculation, coagulation, chemical precipitation, and gravity methods.^{1,21} For extreme heavy metal concentrations (several orders of ppm) the chemical precipitation process, specifically lime precipitation, is the most common and effective pre-treatment step. Despite lower removal efficiency compared to the tertiary treatment process, large quantities of pollutants can be removed using these inexpensive and simple primary processes. Secondary treatment processes are mostly used for organic pollutant removal and are categorized as either anaerobic or aerobic treatments which rely on microorganisms to decompose pollutants into safer substances.¹ Heavy metal biosorbent microbes are being explored for the removal of toxic and valuable metals in industrial wastewater,²² but are not the focus here since this review strives to assess the tertiary treatment technologies for heavy metal removal.

1.4.2 Tertiary Water Purification Technologies

There are several tertiary treatment processes used to remove heavy metals, such as electrochemical precipitation, chemical oxidation, photocatalysis and distillation, with the most common being membrane technologies, ion exchange and adsorption due to their higher removal efficiency for heavy metal ions.^{1,21}

1.4.2.1 Membrane Technologies

Membrane based technologies to purify water can be classified into reverse osmosis, ultrafiltration, nanofiltration, electrodialysis and nano-hybrid membranes. The separation mechanisms of this physicochemical process consist of solution-diffusion, size sieving and the Gibbs-Donnan effect.^{23,24} Although these diverse technologies are simple to fabricate and demonstrate heavy metal ion removal with high efficiency, there is a trade-off between permeability and membrane selectivity. Additionally, the high energy consumption, increased by membrane fouling, and short membrane lifetime due to high pressures is a barrier to the widespread application of pressure driven membrane processes.²⁵

1.4.2.2 Ion Exchange Technologies

The main application of ion exchange technology is to treat drinking water for hardness and can be used to remove heavy metals as well.¹ This physicochemical process is based on a reversible ion-exchange between the heavy metal ions present in the liquid phase with the non-toxic ions presaturating the porous insoluble solid phase.²⁴ The solid phase consists of anion or cation saturated nanoporous resins, and have a high specific surface area. They can be categorized as strong or weak exchangers depending on the functional groups present on the resin, which may be basic or acidic in nature. While the performance of these resins is extremely sensitive to parameters including pH, temperature, initial concentration of the heavy metals in the liquid phase and contact time, an advantage of using this technology is the reversibility of the ion-exchange.¹ Once saturated, the resin can be regenerated via elution with the appropriate reagents, enabling both water purification of heavy metals and recovery of the valuable heavy metals. Furthermore, these resins come in fluidized packed bed configurations that are easy to use, have low energy demand and do not produce secondary pollution. Although there are many advantages to this technology, some central limitations include compulsory resin pre-treatment, highly specific ion-exchange that inhibits simultaneous ion removal, and high operating costs relative to adsorption technologies.¹

1.4.2.3 Adsorption Technologies

Adsorption is another physicochemical method based on mass transfer between the solid phase, called the adsorbent, and the aqueous phase, where the substrate being removed is called the adsorbate.²⁴ Although the terms adsorption, adsorbent and adsorbate are widely used in the literature, this review will refer to adsorption technologies as sorption technologies, where the sorbent is the solid phase and sorbate is the substrate. This terminology is intentionally used to encompass the entire description of this physicochemical process as there are three stages involved in sorption: (i) diffusion of the sorbate from the bulk solution to the sorbent surface; (ii) adsorption of the sorbate onto the sorbent surface; and (iii) absorption/penetration of the sorbate into the sorbent.²⁴ While chapter 2 covers the process and mechanisms of sorption in detail, general parameters which influence sorption include pH, temperature, sorbent dose, sorbate concentration and contact time, and for the column setup there is also bed height and flow rate. While batch experiments are valuable in providing information on the sorbent's binding capacity and sorption mechanism, column experiments mimic the industrial wastewater treatment setup.⁵

From a kinetics perspective, the rate limiting step in the sorption process can vary based on the nature of the sorbent and sorbate, as well as the conditions under which the reaction takes place (i.e. pH, temperature, and initial concentration of the heavy metals in the liquid phase). However, it is commonly accepted that the diffusion of the sorbate to the sorbent surface is often the rate-limiting step, particularly for sorbents with a low specific surface area (SSA).^{2,26} For effective sorption technologies, it is necessary to utilize sorbents with a high SSA which allows for a greater number of accessible sites for sorption, thereby enhancing the kinetics of the overall sorption process.

1.5 Sorbents for Heavy Metal Removal

Advancements in technical performance for various water treatment methods have progressed rapidly, yet considerations of affordability, operational costs and sustainability have not kept pace.¹ Established technologies for the removal of heavy metals from water, such as membrane processes, are typically accompanied by high capital investment and operational costs. Consequently, sorption technology stands out as a preferential method for heavy metal removal from wastewater, underscoring its economic viability, simplicity, and sustainable operations.^{27,28}

The operational sustainability of sorption technology comes from its flexibility to operate in various modes, with both static (batch sorption) and dynamic (column sorption) being economical.¹ The sustainability of materials includes factors such as inexpensive fabrication, adaptability to various characteristics of real wastewater, resiliency to long utilization times/large volumes and environmental safety throughout material lifecycle.⁵

1.5.1 Nanosorbents

Sorption is an effective and economical method for heavy metal removal due to its high removal efficiency, flexibility in design, ease of use, and option to regenerate the sorbents using the appropriate reagent.¹ Since having a high SSA is a primary factor in sorption, nanomaterials are being explored to produce new types of sorbents that have high SSA and high chemical activity. Generally, these are categorized as carbon,²⁴ metal or zeolite-based nanosorbents.²⁹ Activated carbon is a commercial sorbent for wastewater treatment applications, with other emerging carbon-based materials being carbon nanotubes and graphene.^{24,30} Metal-based nanosorbents include various nanometal oxides such as aluminum, magnesium, zinc, titanium and others, where the separation mechanism relies on complexation of oxygen in the metal oxides with the heavy metal ions in water.¹ Zeolites are hydrated aluminosilicate crystalline materials with uniform pore sizes, making them applicable for many applications including heavy metal removal.²⁹ Although nanosorbents present considerable advantages in various applications, notably with their tunable pore sizes, high SSA and accessible binding sites, the utilization of nanomaterials for sorption purposes is accompanied by significant limitations.

Key drawbacks to using nanosorbents include concerns related to safety, potential secondary pollution, and challenges with processing and handling. The safety concerns associated with nanomaterials primarily stem from their unique physicochemical properties, which can lead to unintended toxicological effects on humans and the environment. For instance, nanoparticles can potentially enter biological systems via respiratory or dermal routes, leading to adverse health impacts.³¹ Moreover, the potential for leaching of harmful substances or aggregation in aqueous environments raises concerns regarding secondary pollution, particularly when nanomaterials are used in treatment processes and subsequently released back into the ecosystem.³² Processing and

handling nanomaterials also present challenges due to the small size and reactivity of nanomaterials. These materials exhibit different behaviors compared to bulk counterparts, which can complicate fabricating sorbents and optimizing their performance.^{33,34} Furthermore, the possibility of agglomeration, which can affect the consistency and efficacy of sorbent materials, necessitates specialized techniques for their synthesis, stabilization, and application.^{35,36} In summary, while nanosorbents exhibit remarkable properties for sorption of heavy metals (Table **1.2**), the disadvantages associated with their stability in water, production cost, safety, secondary pollution risk, and processing difficulties must be addressed in future research and applications.²⁰ Table 1.2. Heavy metal sorption capacities of nanosorbents.

Heavy Metals	Nanosorbent	Sorption Capacity (mg•g ⁻¹)	Reference
Pb ²⁺	Multiwall carbon nanotubes/ polyrhodanine nanocomposite	8118	Alizadeh et al. 2016 ³⁷
Cd ²⁺ , Ni ²⁺	Polyvinyl alcohol/ nanozeolite nanocomposite nanofibers	838.7, 342.8	Rad et al. 2014 ³⁸
Cu ²⁺	Polyvinylpyrrolidone-reduced graphene oxide	1689	Zhang et al. 2014 ³⁹
Hg^{2+}	Magnetite single walled carbon nanotubes/ cobalt sulfide nanohybrid	1666	Alijani et al. 2018 ⁴⁰
As ³⁺ , Co ²⁺ , Zn ²⁺	Carbon nanotube coated poly- amidoamine nanocomposite	432, 494, 470	Hayati et al. 2018 ⁴¹
Cr^{3+}	Nitrogen-doped magnetic carbon nanoparticles	638	Shin et al. 2011 ⁴²

1.5.2 Hydrogels

Implementation of nanosorbents, such as biochar and activated carbon, in wastewater management is rising, yet drawbacks include high production cost and low regeneration capacity.^{43,44} Additionally, concerns regarding their ecological and human health impacts remain, necessitating a thorough evaluation of their safety to fully leverage their potential in water treatment.⁴⁵ To overcome these issues, hydrogel sorbents are currently being explored as low-cost and safe nanostructured alternatives which maintain the crucial physicochemical properties needed to effectively remove heavy metals.⁴⁶

Hydrogels consist of hydrophilic cross-linked polymer networks which are insoluble in water and are capable of absorbing water many times their dry weight.⁴⁷ They can be classified based on many characteristics including: polymer composition (synthetic, natural, or hybrid), crosslinking method (physical or chemical), polymer network type (single, semi-interpenetrating, interpenetrating, or polyelectrolyte complexes), charge (non-ionic, cationic, anionic, or ampholytic), pore size (non-porous, microporous, mesoporous, or macroporous), etc.⁵

Hydrogels have been crosslinked via several physical methods, including hydrogen bonds, electrostatic attractions, van der Waals forces, hydrophobic interactions, or metal coordination.^{48–50} Generally, these hydrogels exhibit lower mechanical strength relative to covalently crosslinked hydrogels, which often demonstrate both better mechanical robustness and chemical stability.⁵ This review will focus on covalently crosslinked hydrogels since high strength materials are a prerequisite for many applications, including water remediation. Their ability to swell and retain water enhances their application for environmental remediation, and their substantial surface area can be modified to have a high density of metal ion-coordinating functional groups.⁵¹ Furthermore, the permeability of hydrogels can be fine-tuned by adjusting the crosslinking density,⁵² and their molecular sieving characteristics enable them to operate without needing to pre-treat the water to remove dirt or other contaminants, enhancing their practicality in wastewater applications.

1.5.2.1 Synthetic Hydrogel Sorbent Materials

Various types of synthetic polymers have been successfully employed in making hydrogels for the sorption of heavy metals, including (branched) polyethylenimine,^{53,54} (hydrolyzed) polyacrylamide,^{55,56} polyvinyl alcohol,⁵⁷ and others with water-loving groups. Polyacrylic acidbased hydrogels are particularly notable due to the carboxylate functional groups, which facilitate the sorption process by interacting with positively charged metal ions.⁵⁸ Depending on the functional groups present on the polymer, these materials have been covalently crosslinked through various reactions including click-chemistry, radical polymerization, Schiff base condensation, etc. using a variety of crosslinkers, such as glutaraldehyde,⁵⁷ 3-glycidyloxypropyl trimethoxysilane,⁵³ N,N-methylenebis(acrylamide),⁵⁶ and epichlorohydrin.⁵⁹ However, using small (difunctional) organic and toxic molecules as cross-linkers requires additional washing steps to ensure the crosslinked hydrogel is pure.

Hydrogels have been synthesized from a variety of synthetic and natural polymers, offering a range of functional groups for metal ion interaction.^{5,21} Synthetic hydrogels, while providing superior mechanical strength and enhanced sorption capacities, pose challenges due to the presence of hazardous crosslinking agents and dependence on non-renewable resources.^{51,60} This aspect highlights the importance of exploring natural alternatives and developing non-toxic crosslinking agents to mitigate environmental impacts while leveraging the beneficial properties of hydrogels in wastewater treatment.

1.5.2.2 Bio-renewable Hydrogel Sorbent Materials

Natural polymers have been increasingly investigated as sorbents due to being renewable resources, biodegradable, inexpensive, non-toxic and abundant.^{5,43,57} The various reactive chemical groups, including amines, hydroxyls, carboxylates and sulfate half esters serve as sites for selective interactions or further modification.⁴³ Besides the possibility of high sorption capacity, the wealth of functional groups could be leveraged to engineer hydrogels of various compositions, giving rise to various internal morphologies.⁵

Polysaccharides, found ubiquitously in nature, serve specific functions including energy storage and structural support.⁵ The structure of the polysaccharide dictates its interaction with itself and the surrounding environment. All polysaccharides consist of monosaccharide units (identical or variable) linked by glycosidic bonds, where different polysaccharides have different functional groups located in specific positions on the polymer backbone. Natural sources of polysaccharides can be classified into four main groups: animal (chitin/chitosan, hyaluronic acid, chondroitin sulfate, etc.), bacterial (pullulan, dextran, salecan, xanthan gum, etc.), algal (alginic acid, carrageenan, etc.) and plants (pectin, starch, cellulose).⁵ Several of these polysaccharides have been explored as biosorbents including chitosan,⁶¹ alginate,^{62,63} pectin,⁵⁷ and cellulose,^{26,50} as they are inexpensive, easy to extract from raw materials, and commercially accessible.⁵ The metal sorption capacity of bio-based hydrogel composites is shown in **Table 1.3**. While many polysaccharides offer competitive binding capacity for hydrogels, this review will focus on cellulose, the most easily degradable and abundant structural biopolymer on Earth found in plant and non-plant sources.⁶⁴
Heavy Metals	Bio-based Hydrogel Composites	Sorption Capacity (mg•g ⁻¹)	Ref.
Cd ²⁺ , Co ²⁺ , Cu ²⁺ , Ni ²⁺ , Pb ²⁺	Glucan/chitosan/acrylic acid hydrogel crosslinked using N'N-methylenebisacrylamide	269, 232, 342, 184, 395	Jiang et al. 2019 ⁶⁵
Cu^{2+}	Magnetic (encapsulated Fe ₃ O ₄) calcium alginate coated chitosan hydrochloride beads	143	Yi et al. 2018 ⁶⁶
Cu ²⁺	Cellulose nanofiber from almond shell crosslinked with sodium trimetaphosphate	148	Maaloul et al. 2021 ⁶⁷
Cu^{2+}, Pb^{2+}	Sodium alginate grafted polyacrylamide/ graphene oxide hydrogel	69, 241	Jiang et al. 2020 ⁶⁸
Cr^{6+}, Cu^{2+}	Chitosan/Fe ⁰ nanoparticle/carboxymethyl/β- cyclodextrin beads	200, 250	Sikder et al. 2014 ⁶⁹
Cd ²⁺ , Cu ²⁺ , Pb ²⁺	Ethylenediaminetetra-acetic acid cross- linked chitosan/N,N- methylenebis(acrylamide) cross-linked polyacrylamide double network hydrogel	86, 99, 138	Ma et al. 2017 ⁷⁰
Cu ²⁺ , Mn ²⁺ , Pb ²⁺	Magnetic (encapsulated Fe ₃ O ₄ nanoparticles) sodium alginate/ carboxymethyl cellulose composite hydrogel	106, 72, 89	Wu et al. 2021 ⁶³
Co ²⁺ , Cu ²⁺ , Pb ²⁺ , Zn ²⁺	Sodium acrylate-co-N-isopropylacrylamide based pectin hydrogel	52, 54, 55, 50	Thakur et al. 2019 ⁵⁷
Cd ²⁺ , Ni ²⁺ , Pb ²⁺	Cellulose-graft-acrylic acid hydrogel	563, 380, 826	Zhou et al. 2012 ⁷¹
Pb^{2+}, Zn^{2+}	Carboxymethyl cellulose-graft-poly(acrylic acid)/montmorillonite hydrogel	146, 287	Astrini et al. 2015 ⁷²
Pb ²⁺	Carboxylated cellulose nanocrystal/sodium alginate hydrogel beads	339	Hu et al. 2018 ⁷³
Pb^{2+}	Carboxylated chitosan/carboxylated nanocellulose hydrogel beads	335	Xu et al. 2021 ⁷⁴
Pb ²⁺	Cellulose nanofiber/sodium alginate hydrogel beads	318	Zhao et al. 2021 ⁷⁵
Cu ²⁺ , Zn ²⁺ , Ni ²⁺	Glutaraldehyde cross-linked polyethylenimine-grafted pea protein sponges	67, 116, 56	Zhu et al. 2025 ⁷⁶
Cd ²⁺ , Cu ²⁺ , Pb ²⁺	Carboxymethyl cellulose/pectin-based hydrogel beads crosslinked with chitosan	139, 144, 270	Zhang et al. 2025 ⁷⁷

Table 1.3. Heavy metal sorption capacities of bio-based hydrogels.

1.6 Cellulose as a Bio-renewable Material for Heavy Metal Sorption

Cellulose is a non-toxic, biocompatible, renewable and biodegradable biopolymer with a linear semicrystalline structure with three distinct regions: a nonreducing end, repeating cellobiose units, and a reducing end (Figure 1.3).⁷⁸ More specifically, cellulose is composed of D-glucose units linked by ß-1,4 glycosidic linkages with repeating cellobiose units.^{64,79} Cellobiose is a dimer of anhydrous glucose units (AGU), where the AGUs are rotated 180° with respect to each other. The cellulose degree of polymerization (DP) is defined by the number of AGUs, not the number of cellobiose units in each glucan chain. Each AGU has one methylol group (-CH₂OH) at C-6, and two secondary hydroxyl groups (-OH) at C-3 and C-4.80 Although hydroxyls are hydrophilic functional groups, cellulose is not soluble in water due to the dense network of intra-/intermolecular hydrogen bonding facilitated by the hydroxyl groups and van der Waals forces between the glucose units.⁸¹ In addition to the insolubility of cellulose in water, the hydroxyl groups only become charged at pH above 10, thus cellulose is not directly applicable for developing hydrogels for sorption of heavy metals which require charged functional groups. Fortunately, due to the low-cost, abundance, and widespread pertinency of cellulose in biomedical, pharmaceutical, food and agriculture applications, the chemical modification of cellulose to produce soluble derivatives has been studied extensively.^{79,80}



Figure 1.3. Chemical structure of cellulose depicting the nonreducing end, repeating cellobiose units — which consist of a dimer of anhydrous glucose units rotated 180° with respect to each other, and reducing end. Reproduced from Habibi *et al.*⁷⁸

1.6.1 Cellulose Extraction

Cellulose can be derived from various sources, such as plants, animals and microbes where it primarily serves as a structural material. From the variety of sources — examples being grasses (bagasse, bamboo), seed fibers (cotton), bast fibers (flax, hemp, jute, ramie), wood fibers (hardwoods and softwoods), algae (*Valonia ventricosa*), and bacteria (*Acetobacter xylinum*) — commercial production of cellulose is done primarily using cotton and wood as the raw materials, which contain 90-99% and 40-50% cellulose content, respectively.⁸⁰ During cellulose extraction from any raw material, other components such as lignin and hemicellulose must be removed. This involves three steps: (1) Pre-hydrolysis treatment with alkali or mineral acid; (2) pulping by cooking the fibre in alkali conditions such as NaOH; and (3) bleaching with sodium chlorite, hydrogen peroxide, ozone, etc.⁸² For the extraction of cellulose from wood pulp, the kraft pulping process is used, where wood chips are treated with a hot aqueous mixture of sodium hydroxide and sodium sulfide, which breaks the bonds that link cellulose, lignin and hemicellulose. This pure cellulose can be further modified to introduce functional groups that yield water-soluble cellulose derivatives.⁸⁰

1.6.2 Cellulose Modification

To enhance the solubility of cellulose in water, the abundance of hydroxyl groups on cellulose (three per AGU) can be modified, which would disrupt the hydrogen bonding that is responsible for the insoluble crystalline regions and enable further modification to introduce crosslinking or metal chelating functional groups.⁷⁹ Cellulose can be modified through various methods which can be categorized as monomer-grafting or direct chemical modification.⁸⁰ While a range of versatile functional groups can be attached to the cellulose backbone through various monomer-grafting reactions (photografting, high energy radiation grafting, and chemical initiation grafting), this section focuses on direct chemical modification methods where the functional groups are attached to the hydroxyl groups of cellulose. Various routes of direct cellulose modification include oxidation, halogenation, etherification, esterification, etc.^{1,2} with cellulose ethers being widely used compared to other cellulose derivatives.⁷⁹

To obtain cellulose ethers, the first step is forming alkali cellulose using aqueous sodium hydroxide solution. Then, through the etherification process (typically with O-alkylation with alkyl halides or epoxide addition), side groups are incorporated into the cellulose backbone.⁷⁹ The general reaction steps are shown, with R being the AGU and R* being the ether side group:

- (1.1) ROH + NaOH \rightarrow RONa (alkali cellulose) + H₂O
- (1.2) RONa + R*Cl \rightarrow ROR* + NaCl

Depending on the side group (R*) and degree of functionality (DOF), the cellulose ether derivative has unique properties which dictate its solubility and uses. Common cellulose ether derivatives include methyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, carboxymethyl cellulose sodium salt, hydroxyethyl cellulose and ethyl cellulose.⁷⁹ The industrial production of cellulose ethers began in the 1920s owing to their non-toxicity, good solubility and

high chemical stability, which make them valuable additives for drilling technologies, building materials, pharmaceuticals, cosmetics and the food market .⁶⁴ As additives to drilling fluids, they retain rock dust in suspension, stabilize the bore and keep the drilling head cool. In the building materials market, they are admixed to mortar to control the consistency in the processing and rheology of plaster systems. In pharmaceuticals, cosmetics and the food market, cellulose ethers are useful since they bind well to water, are stable in freeze/thaw cycles, mediate liquid viscosities, and are odorless and tasteless.⁶ Furthermore, these cellulose ether derivatives have been used to make hydrogels for various applications, such as wound healing,^{83,84} insulin delivery,⁸⁵ wearable sensors,⁸⁶ agriculture,⁸⁷ and wastewater treatment.^{88,89}

For the application of water purification of heavy metals, carboxymethyl cellulose (CMC) is of particular interest due to its abundant hydroxyl and carboxylate groups. The sorption efficiency of sorbents depends largely on the number and availability of electron-rich functional groups present in the sorbent.⁵ While the carboxylate functional groups provide CMC its inherent ability to chelate metal ions, the hydroxyl groups can be modified to enable crosslinking to generate stable hydrogel networks. Although CMC is the ideal derivative to use for making hydrogels for metal-chelation, the scarcity of AGUs (only 10-35 % of the polymer chain) — which have primary hydroxyls available for further chemical modification — hinders the chemical ability to introduce reactive crosslinking functional groups onto CMC. Due to this limitation and the need to have a high degree of functionality (DoF) of crosslinking functional groups on the cellulose backbone for it to covalently crosslink and form a hydrogel, other cellulose derivatives must be explored. Thus, hydroxyethyl cellulose (HEC), which has primary hydroxyl groups available on the entirety of the polymer backbone, was modified alongside CMC to form covalently crosslinked metal-chelating hydrogels. The modification, characterization and hydrogel properties are explored in Chapter 2.

1.6.3 Cellulose Nanocrystals

Cellulose based hydrogels for water purification of heavy metals are currently being explored in bulk formats,^{62,63,90,91} with batch experiments being done to obtain essential information on the sorbent's binding capacity and sorption mechanism. While this is acceptable at the research and development phase, to employ hydrogels at the industrial scale, hydrogels should be templated into microbeads which can fill a column to be transferrable to existing industrial operations and equipment.⁵ The bead format requires the hydrogel to be both flexible and mechanically robust. While biopolymers like HEC and CMC impart flexibility to the hydrogels, these hydrogels remain fragile for bead templating, handling, and dynamic use in a column format. Thus, nanocellulose offers a promising renewable and biodegradable solution to enhance the mechanical robustness of cellulose based hydrogels while continuing to implement the principles of green chemistry.

Nanocelluloses are divided into three categories: bacterial nanocellulose, produced by bacteria or biotechnologically; cellulose nanofibrils, produced mechanically by delaminating plant-based cellulose; and cellulose nanocrystals (CNCs), isolated by chemical hydrolysis or oxidation.⁸¹ Due to their fascinating intrinsic properties, CNCs have served as superior reinforcing materials in the nanocomposites field, and have been incorporated into various synthetic and biopolymer matrices.^{78,92} Some of these properties include high mechanical strength — elastic modulus of 143 GPa, comparable to Kevlar (60-125 GPa) and approaching steel (200-220 GPa), low density (1.61 g/cm³ for CNC vs 8 g/cm³ for steel), and high specific surface area.⁹²

The cellulose derivatives employed to make hydrogels (such as HEC and CMC covered in Chapter 2) consist of single glucan chain polymers, while CNCs are rigid nanoparticles produced from elementary fibrils which consist of approximately 36 glucan chains.⁷⁸ Elementary fibrils have both crystalline (ordered) and amorphous (disordered) regions, which arise from the numerous

hydrogen bonds and internal strain between packed chains, respectively.⁷⁸ The disordered regions are present at regular intervals and loosely packed, making them easily accessible for chemical degradation (**Figure 1.4**).⁷⁹ Upon sulfuric acid hydrolysis — most notably used to produce CNCs at an industrial scale, the amorphous regions get hydrolyzed selectively, resulting in highly crystalline (54 - 99%) rod shaped CNCs with high-aspect ratios.^{78,81} CNCs derived from plant celluloses have lengths ranging from 100-250 nm and cross sections of 5-70 nm, depending on the plant source and extraction method.⁸¹ Additionally, when produced using sulfuric acid hydrolysis, an esterification reaction results in some surface hydroxyl groups on CNCs to be substituted by anionic sulfate half-ester groups, which promote colloidal stability of CNC suspensions in water.⁸¹ In addition to these anionic groups, the abundance of primary hydroxyl groups on the surface can allow for modifications to add both crosslinking functionality (ex. aromatic aldehydes) and metal chelating functional groups (such as carboxylates and amines).



Figure 1.4. Depiction of cellulose nanocrystal (CNC) fabrication via acid hydrolysis of semicrystalline (crystalline and amorphous region) cellulose fibers. Reproduced from Bhaladhare *et al.*⁷⁹

Composite hydrogels have demonstrated superior sorption capacities due to the presence of ioncoordinating sites that engage directly with heavy metal ions.⁴⁶ Incorporation of modified CNCs in a composite hydrogel would ideally increase both cross-link density and binding capacity. Chapter 3 explores unmodified and modified-CNCs to understand how their incorporation into a covalently crosslinked cellulose hydrogel network can enhance the hydrogel's mechanical strength. The increased crosslinking alongside the CNC's intrinsic mechanical strength would produce robust composite hydrogel materials, required for both microbead templating for column use and potential reusability — desired features from both an economic and sustainability perspective, and requirements for transitioning to industrial scale applications.

1.7 Conclusion

Water plays a vital role in human physiology, supporting essential bodily functions and serving a wide range of purposes, from agriculture to cleaning, food processing, and chemical manufacturing. Therefore, managing water resources is paramount to ensure ecological sustainability and human welfare. In the context of enhancing water purification technologies globally, the need to prioritize accessible, affordable and sustainable water purification materials is necessary. Although efficient, many commercial sorbents are powders that are hard to process, are not recyclable or fully biodegradable, or are costly. To address this challenge, this thesis explores the development of a cost-effective, bio-renewable, and biodegradable hydrogel for heavy metal sorption, made following the principles of green chemistry (7-use of renewable feedstock and 10-design for degradation). This chapter provided context on major water pollutants and water purification technologies, introduced sorption technology and sorbent materials to address heavy metal pollution, discussed the need for hydrogels as alternatives to current powdered sorbents and examined cellulose in detail as the biomaterial of choice to undertake the goal of addressing the need for environmentally benign heavy metal binding hydrogel sorbent materials. Chapter 2 covers the investigation of cellulose derivatives used to make bio renewable hydrogels, which are characterized and optimized for their crosslinking ratio and composition; evaluated for their

swelling and stability in different environmental conditions, and tested for their physicochemical properties including sorption isotherms, sorption kinetics, and a multi-metal sorption study for metal ions (Cu²⁺, Zn²⁺, Co²⁺, Ni²⁺ and Mg²⁺). The findings from Chapter 2 were used to address the goals covered in Chapter 3, which primarily focused on developing more robust hydrogels which can be employed at an industrial scale one day. Briefly, this included exploring the incorporation of pristine and modified-cellulose nanocrystals as a green nano-additive into the hydrogels developed in Chapter 2. These hydrogel composites were tested for their swelling, mechanical strength and binding capacity. Finally, Chapter 4 summarizes the key findings from Chapter 2 and 3 alongside next steps to work towards addressing the need for industrially employable, sustainable, and robust sorbents for heavy metal sorption made with the principles of green chemistry. Overall, this work showcases how cellulose-based hydrogels have the potential to address the need for inexpensive, accessible, and renewable sorbents for water purification of heavy metals at a global scale.

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Chapter 2: Tetrahydrazide-EDTA-Crosslinked Cellulose Hydrogels for Water Treatment by Heavy Metal Chelation

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Chapter 2 TOC Figure Caption. Hydrogels form as aldehyde groups on aa-HEC and aa-CMC (purple moiety on polymer strands) and hydrazide groups on 4h-EDTA (blue corners on black star) react to form hydrazone bonds. Metal (Cu²⁺, cyan star) chelation by the available coordinating groups on the aa-CMC and 4h-EDTA give the hydrogel a cyan colour. Structures not drawn to scale.

Abstract

Polluting heavy metals persist in the environment, leading to bioaccumulation and toxicity, a growing problem in developing countries. Various water filtration systems for heavy metal removal have been developed, with sorption being the simplest and most economically viable. However, many commercial sorbents are powders leading to inefficient sorbent removal and secondary pollution. Our goal was to develop renewable, biodegradable, and cost-effective hydrogel sorbents able to bind heavy metals. This was accomplished using hydroxyethyl cellulose (HEC) and carboxymethyl cellulose (CMC) functionalized with aromatic aldehydes (aa-HEC and aa-CMC), and an ethylenediaminetetraacetic acid (EDTA)-based crosslinker modified with four hydrazide groups (4h-EDTA). By varying the ratio of aldehyde-to-hydrazide (a:h) groups in the aa-HEC/4h-EDTA hydrogel, a ratio of 1:2 a:h was found to have the maximum storage modulus (G') and was used to make 25/75 aa-HEC/aa-CMC 2 wt% aa-HEC/aa-CMC/4h-EDTA hydrogels, with a G' of 200 Pa and a maximum sorption capacity of 102 mg of Cu^{2+} per gram of hydrogel. The sorption capacity of the hydrogels was tested for Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺ and Mg²⁺ individually and as a multi-metal mixture, with Cu²⁺ showing the highest affinity. This work shows that cellulose-based hydrogels could be used as a green alternative for the removal of heavy metal pollutants from water.

2.1 Introduction

Potable water is essential to every community, yet water scarcity due to pollution by heavy metals is a growing problem severely impacting resource-limited areas where drinking water is already scarce.^{1,2} Point sources for pollution include mining and discharge of industrial waste, while non-point sources include construction, urban run-off, and leaching from landfills.³ In fact, the increasing demand for consumer electronics is generating large amounts of electronic waste (e-waste), which is one of the fastest growing contributors of heavy metal pollution in water systems.⁴ Heavy metals — such as Cu²⁺ and Ni²⁺ ions leached from e-waste — persist in the environment and can lead to bio-accumulation and toxicity.⁵ Although Cu²⁺ is an essential element for human health, excess Cu²⁺ can negatively affect the heart, kidneys, liver, and cognitive functions.⁵

To combat the problem of heavy metal pollution, various filtration systems have been developed, like ion exchange, chemical precipitation, nano-filtration, electrodialysis, and sorption, with the latter being the simplest and most economically feasible.⁶ Commercial sorbents for water treatment include biochar and polymers;⁷ however, many are powders that lead to low sorbent removal efficiency and recyclability, as well as secondary pollution.⁶ To overcome these issues, metal-organic frameworks (MOFs) and polymeric hydrogels are currently being explored. MOFs, and more generally nanoparticle ion scavengers, have been shown to have high binding capacities resulting from their high density of surface-active sites, but have found limited practical use due to their high cost and powder form, making them harder to process and handle.^{8,9,10}

Alternatively, hydrogels are three-dimensional, crosslinked, hydrophilic polymeric networks that can absorb water up to thousands of times their dry weight.¹¹ Hydrogels are promising as heavy metal scavengers because their large internal surface area can be modified to have a high

density of metal ion-coordinating groups.¹² Additionally, hydrogel permeability can be controlled via crosslinking density,¹³ and their molecular sieving effect eliminates the need to pre-treat water for dirt and other environmental contaminants.¹⁴ Synthetic and synthetic-natural composite hydrogels have been explored as sorbents for heavy metal removal. For example, a synthetic hydrogel made using hydrolyzed polyacrylamide and branched polyethylenimine resulted in high mechanical strength (storage modulus of 1093 Pa) and high binding capacity (482.2 mg/g for a mixture of metal ions and 436.5 mg/g for Cu²⁺).¹⁵ Composite sorbents have been made from polyethylenimine and cellulose nanofibrils crosslinked using 3-glycidyloxypropyl trimethoxysilane,¹⁶ a double network of ethylenediaminetetraacetic acid (EDTA) crosslinked chitosan and N,N-methylenebis(acrylamide) crosslinked polyacrylamide,¹⁷ and carboxymethyl cellulose (CMC) and polyacrylamide crosslinked via free-radical polymerization using N,N'methylenebis(acrylamide).¹⁸ Furthermore, some CMC based hydrogels include hydrogel beads crosslinked using epichlorohydrin (sorption capacity of 412.4 mg/g for Cu²⁺),¹⁹ a CMC/polyvinyl alcohol/cellulose nanocrystal hydrogel (109.89 mg/g for Cu²⁺),²⁰ and a sodium alginate/CMC with in-situ Fe₃O₄ nanoparticles composite hydrogel (105.93 mg/g for Cu²⁺).²¹ Although synthetic or composite hydrogels offer high mechanical strength and high sorption capacity for heavy metals, there are drawbacks such as the use of toxic crosslinkers and the reliance on non-renewable and non-biodegradable resources.²² Thus, the overarching goal of this work was to create a costeffective hydrogel able to efficiently chelate heavy metals, while implementing the principles of green chemistry (7-use of renewable feedstock and 10-design for degradation).²³

Cellulose, a polymer of β-1-4 linked anhydroglucose units, is the most abundant bio-renewable polymer on Earth.²⁴ Although cellulose has poor dispersibility as a result of the many hydroxyl groups along its backbone forming inter and intramolecular hydrogen bonds, these hydroxyl

groups can be modified to introduce crosslinking and metal binding sites, and enable easy dissolution in water and other common solvents.¹² The studies described above show that CMC can serve as an excellent hydrogel component for the sorption of heavy metals. CMC is a commercial cellulose derivative with amphiphilic character due to the hydrophobic backbone and hydrophilic/negatively charged carboxylate pendant groups.²² In this work, a bio-renewable and biodegradable hydrogel was made using CMC as the main cellulose derivative that efficiently binds heavy metals, and hydroxyethyl cellulose (HEC), which acts as a backbone that provides structural support. Both cellulose derivatives were functionalized with aromatic aldehyde groups (aa-HEC and aa-CMC). These were crosslinked by an EDTA derivative bearing four hydrazide groups (4h-EDTA) that react with aldehydes to form dynamic hydrazone bonds (Figure 2.1). An interesting feature of the 4h-EDTA crosslinker is its ability to serve as a coordinating site for a metal-ion along with the carboxylate groups on the aa-CMC. The main objective of this work was to use green constituents aa-HEC/aa-CMC (H/C) crosslinked with 4h-EDTA to form stable hydrogels that could efficiently bind heavy metals from water. The optimal aa-HEC/aa-CMC/4h-EDTA hydrogel was found to contain a 1:2 a:h ratio, a 25/75 H/C proportion, and had a maximum sorption capacity of 102 mg of Cu²⁺ /g of dry hydrogel. Our hydrogels show that cellulose derivatives can be crosslinked using an environmentally benign crosslinker to make bio-renewable and biodegradable sorbents for sustainable water purification of heavy metals. Moving forward, these cellulose-based hydrogels contribute to understanding the mechanical and physicochemical properties of green sorbents and serve as a basis for further hydrogel development and implementation as cost-effective and sustainable alternatives to synthetic and powdered sorbents that are not sustainable and are difficult to handle/recover.



Figure 2.1. Chemical and experimental overview. aa-HEC and aa-CMC crosslinked by 4h-EDTA via hydrazone bonding between the aromatic aldehyde (purple groups on polymer strands) and hydrazide (blue tips on black star) groups, respectively, where remaining hydrazide groups could chelate the metal (cyan star). Structures are not drawn to scale.

2.2 Experimental Section

2.2.1 Materials

The following chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada) and used without further purification: 2-hydroxyethyl cellulose (HEC) (90,000 Da; lot number: MKCJ9416), sodium carboxymethyl cellulose (CMC) (90,000 Da; lot number: MKCM6314; 0.7 carboxymethyl groups per anhydroglucose unit), vanillin, tetrahydrofuran (THF), cyanuric chloride, ethylenediaminetetraacetic acid (EDTA), ethanol (EtOH), sulfuric acid (H₂SO₄), dichloromethane (DCM), sodium bicarbonate (NaHCO₃), 50-60 wt% hydrazine hydrate, sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium citrate dihydrate (C6H5Na3O7·2H2O), citric acid monohydrate (C6H8O7·H2O), dibasic sodium phosphate (Na2HPO4), monobasic sodium phosphate (NaH2PO4), sodium carbonate (Na2CO3), sodium chloride (NaCl), sodium azide (NaN_3) , sodium acetate (CH₃COONa), acetic acid (CH₃COOH), copper(II) sulfate pentahydrate (CuSO4·5H2O), nickel(II) chloride hexahydrate (NiCl2·6H2O), cobalt(II) chloride hexahydrate (CoCl₂·6H₂O), magnesium chloride hexahydrate (MgCl₂·6H₂O), zinc nitrate (Zn(NO₃)₂), and poly(ethyleneimine) solution (PEI) (average Mn ~60,000 by GPC, average Mw ~750,000 by LS, 50 wt% in H₂O). Phosphate buffered saline (PBS) 10x was purchased from BioShop (Burlington, ON, Canada). FluoSpheres, 1.0 µm yellow-green, fluorescent (505/515) microspheres, 2% solids in distilled water, 2 mM azide, were purchased from Invitrogen (Eugene, OR, USA). Milli-Q water with a resistivity of 18.2 MΩ cm (Milli-Q Advantage A10 Water Purification system, Millipore Sigma, Etobicoke, ON, Canada) was used for all experiments, and is subsequently referred to simply as "water".

2.2.2 Synthesis of aa-HEC & aa-CMC

The chemical grafting of vanillin onto HEC was conducted using a one-pot synthesis procedure (Appendix A, Scheme A1). Briefly, under vigorous stirring using an IKA RW 20 digital overhead stirrer (IKA Works, Inc., Wilmington, NC, USA), 0.23 g (5.75 mmol) of NaOH and 0.29 g (1.91 mmol) of vanillin were left to dissolve in a solution of 1 g of HEC dissolved in 50 mL water for 1 h until a clear and faint yellow solution was generated. To this stirring solution, 10 mL THF was added dropwise over 10 min, followed by dropwise addition of 0.35 g (1.90 mmol) cyanuric chloride dissolved in 2 mL THF over a course of 1 min. The reaction was allowed to proceed under vigorous stirring for 1 h, with an additional ~10-15 mL THF added slowly over the first 30 min to maintain a 7:3 water: THF ratio as the THF was lost due to evaporation while using the overhead stirrer. Modified aa-HEC was centrifuged twice at 12,000g for 10 min each time, and the supernatant was purified by dialysis with a membrane (MWCO = 14 kDa) in water for 1 week. The dialyzed aa-HEC solution was centrifuged again (10 min at 12,000g) and freeze-dried using a SP VirTis BenchTop Pro with Omnitronics freeze drier (Warminster, Pennsylvania, USA) for 48 h for storage and subsequent use at 2 wt%. This protocol was repeated to prepare aa-CMC using: 2 g of CMC dissolved in 100 mL water, 0.276 g of NaOH, 0.34 g of vanillin, and 0.42 g of cyanuric chloride. The same solvent ratio (7:3 water:THF, 30 mL THF added slowly into the 100 mL solution, followed by dropwise addition of cyanuric chloride dissolved in 10 mL THF) and cleanup steps were used prior to freeze drying and preparation of a 2 wt% stock solution. All nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AV600-600 MHz NMR spectrometer. ¹H NMR (600 MHz, D₂O, ppm) δ: 7.63 (m, 3 H, CH), 9.88 (s, 1 H, OCH) for aa-HEC (Appendix A, Figure A1A), and 7.62 (m, 3 H, CH), 9.88 (s, 1 H, OCH) for aa-CMC (Appendix A, Figure A1B). All Fourier Transform Infrared Spectroscopy (FTIR) spectra,

discussed later, were recorded on a Thermo Nicolet 6700 FTIR spectrometer in transmission mode using KBr disks loaded with freeze dried samples.

2.2.3 Quantification of Aldehyde Content on aa-HEC & aa-CMC

Absorption spectra of vanillin, unmodified and modified HEC and CMC were recorded by Tecan i-Control software between 230–420 nm using an Infinite 200 plate reader (Tecan, Morrisville, North Carolina, USA) and a Greiner UV-Star 96 well plate (VWR International, Mississauga, ON, Canada) with 200 μ L samples/well, where a λ_{max} of 310 nm was found. A vanillin calibration curve (0-60 μ M) was plotted using corrected absorbance values (using water as the blank) at λ_{max} = 310 nm (**Appendix A, Figure A2**). The modified (aa-HEC and aa-CMC) and control (HEC and CMC) samples were prepared in triplicate as 0.05 wt% solutions and the corrected absorbance (using respective control as the blank) was used to calculate the degree of functionality.

2.2.4 Synthesis of 4h-EDTA

The small molecule crosslinker was synthesized in two steps (**Appendix A, Scheme A2**). First, EDTA (10 g, 34.22 mmol) was added into a round bottom flask containing 150 mL ethanol. Concentrated H₂SO₄ (6.4 mL, 0.120 mol) was added dropwise while the solution was continuously stirred. The reaction mixture was then refluxed at 90 °C overnight (~17 h) with a nitrogen gas balloon. Thereafter, the reaction mixture was concentrated under vacuum to give a colorless viscous oil as product. The oil was removed into a large (500 mL) Erlenmeyer flask, using DCM, and filled to a total volume of 150 mL DCM. To prepare the acid-free product, excess NaHCO₃ solution (15 g in 120 mL, 0.179 mol) was added dropwise while stirring until bubbling stopped. The resulting aqueous and DCM solution was allowed to phase separate. The DCM phase was extracted with water and dried using MgSO₄. The anhydrous DCM phase was then filtered and

concentrated under vacuum to give an oil – EDTA tetraethyl ester. Yield (10.61 g, 26.23 mmol, 76.7%). ¹H NMR (600 MHz, CDCl₃, ppm) δ: 1.26 (t, 12 H, OCH₂CH₃, J = 7.2 Hz), 2.98 (s, 4 H, N-CH₂), 3.66 (s, 8 H, COCH₂N), 4.15 (q, 8 H, OCH₂CH₃, J = 7.2 Hz). ¹³C NMR (151 MHz, CDCl₃, ppm) δ: 14.17 (OCH₂CH₃), 52.10 (N-CH₂), 55.17 (COCH₂N), 60.64 (OCH₂CH₃), 170.90 (CO) (Appendix A, Figure A3).

To synthesize 4h-EDTA (tetrahydrazide-EDTA), EDTA tetraethyl ester (1.5 g, 3.71 mmol) was added into a round bottomed flask containing ethanol (6.5 mL), then hydrazine hydrate (2.03 mL, excess) was added dropwise while stirring continuously. The reaction mixture was refluxed at 100 °C over the course of 4 h. The resulting mixture was triturated and washed using chilled EtOH to give white powder as the final product. Yield (1.22 g, 94.4%). ¹H NMR (600 MHz, D₂O, ppm) δ : 2.67 (s, 4H, NCH₂CO), 3.30 (s, 8H, N-CH₂). ¹³C NMR (151 MHz, D₂O, ppm) δ : 52.70 (NCH₂), 56.80 (N-CH₂CO), 171.84 (CO) (Appendix A, Figure A4).

FTIR spectra of EDTA, EDTA tetraethyl ester and tetrahydrazide-EDTA were also recorded (**Appendix A, Figure A5**). Mass spectra were recorded on a Micromass Quattro Ultima (ESI/APCI-LCMS Triple Quadrupole Mass Spectrometer) (Waters, Milford, MA, USA) that used electrospray ionization. ESI-MS (M+H)⁺ of EDTA tetraethyl ester: 405.3, Na-adduct: 427.3 (**Appendix A, Figure A6A**). ESI-MS (M+H)⁺ of tetrahydrazide-EDTA: 349.3, Na-Adduct: 371.3 (**Appendix A, Figure A6B**).

2.2.5 Fabrication of Hydrogel Pucks & Monoliths

A buffered 4h-EDTA stock solution was prepared by dissolving 4h-EDTA into 1x PBS buffer, with pH adjusted to 7 using 1 M HCl. Initially, 2 wt% aa-HEC pucks were templated using various aldehyde-to-hydrazide (a:h) ratios by mixing 2 wt% aa-HEC with 4h-EDTA stock solution diluted to 30 μ L with 1x PBS to obtain a:h ratios of 4:1, 1:2, 1:1, 1:2 and 1:4. The mixtures were vortexed

for 30 s and $\sim 200 \ \mu\text{L}$ were pipetted into PDMS moulds (diameter = 8.9 mm and height = 3 mm). Although the gelation time depended on the a:h ratio, all pucks were left in the mould for 24 h at room temperature in a closed hydrated environment before removing for rheological measurements.

The optimal a:h ratio, as determined through rheology, was then used to make aa-HEC/aa-CMC/4h-EDTA pucks similar to the aa-HEC/4h-EDTA pucks, where the only difference was varying the volume of aa-HEC in the total volume of the hydrogel to give different proportions of aa-HEC to aa-CMC. The optimal aa-HEC/aa-CMC (H/C) proportion, as determined through a compromise of mechanical robustness and binding capacity, was then used for the rest of the experiments. The optimal system was used to make 2 wt% hydrogel monoliths (150 μ L in volume) in 2 mL microcentrifuge tubes for physicochemical studies by pipetting the appropriate amounts of each component, in the order aa-HEC ® aa-CMC ® 4h-EDTA into a microcentrifuge tube and vortexing for 30 s after each addition, and then capping and leaving to gel overnight prior to use.

2.2.6 Rheological Tests

Hydrogel samples were subjected to a shear test and a frequency sweep using a Discovery Hybrid Rheometer (DTH-20, TA instruments, Mississauga, Ontario, Canada). First, shear tests were performed on aa-HEC/4h-EDTA crosslinked pucks 24 h after templating from 0.1% to 100% strain at a frequency of 10 rad/s (**Appendix A, Figure A7A**). A 0.6% strain was chosen to ensure samples were deformed within the linear region. Then frequency sweeps were performed on the pucks and on in-situ gelled material at 25°C, with strain of 0.6%, angular frequency of 0.1-100 rad/s and 5 points per decade (**Appendix A, Figure A7B-D**). To prepare the in-situ gelation mixture, 600 μ L of 2 wt% aa-HEC was vortexed for 10 s with 30 μ L of the corresponding 4h-EDTA stock — to obtain a:h ratios of 4:1, 2:1, 1:1, 1:2 and 1:4 — prior to pipetting 200 μ L aliquots onto the Peltier plate. The storage (G') and loss (G") moduli were determined for both the premade pucks and the in-situ gelled materials at all five aldehyde:hydrazide (a:h) ratios by taking the average of a fixed range of frequency values (1-10 rad/s), and averaging over three independently prepared samples.

The optimal a:h ratio was used to template aa-HEC/aa-CMC/4h-EDTA pucks to obtain H/C proportions of 100/0, 75/25, 50/50, 25/75 and 0/100. Frequency sweeps and data analysis to determine the G' and G" was performed as described above for the aa-HEC pucks; data analysis took the average of a 0.2-2 rad/s range of frequency values (**Appendix A, Figure A8A-B**). The same conditions were used to perform rheological tests on 2 wt% pucks of the optimal H/C system after incubating them in three different environments for 24 h: (1) 10 mM pH 7 phosphate buffer with 0 mM NaCl; (2) 10 mM pH 7 phosphate buffer with 100 mM NaCl; and (3) excess Cu²⁺ solution (208 mg Cu²⁺/g hydrogel) (**Appendix A, Figure A8C-D**). Note: Due to the change in the puck size during incubation in the three different environments, the moulds used to template these pucks were adjusted to yield a similar final diameter of ~9 mm. This was done to ensure a direct comparison could be made of the robustness obtained for the various conditions relative to the non-incubated pucks, where the diameter is the critical parameter to maintain consistent since that is the surface which contacts the rheometer plate.

2.2.7 Hydrogel Stability

The long-term stability of the optimal aa-HEC/aa-CMC/4h-EDTA hydrogel system was tested in acidic, neutral and basic buffer solutions and in neutral buffer solutions with increasing ionic strength (0-100 mM NaCl) over a 6-week period. 10 mM citrate buffer was prepared with the addition of sodium azide to 0.02 wt% and adjusted to pH 3 using 1 M HCl. 20 mM phosphate buffer was prepared with the addition of sodium azide to 0.04 wt% and adjusted to pH 7 using 1
M NaOH. 10 mM carbonate buffer was prepared with the addition of sodium azide to 0.02 wt% and adjusted to pH 11 using 1 M NaOH. The citrate and carbonate buffers were used as prepared, while the phosphate buffer was diluted 2-fold with water and/or various volumes of 200 mM NaCl stock to obtain 10 mM pH 7 phosphate buffer with 0, 10, 50 and 100 mM NaCl concentrations, thus allowing the puck stability to be monitored in a neutral environment with varying ionic strengths. The sodium azide was added as a proactive measure to prevent bacterial growth in the buffers over the six weeks stability study.

Pucks of the optimal aa-HEC/aa-CMC/4h-EDTA hydrogel composition were made as described earlier, but the hydrogel mixture was prepared with the addition of amine-modified Fluospheres (0.01% solids in total bulk hydrogel volume) to allow clear imaging of the puck area. After 24 h at room temperature, the pucks were removed from the moulds, weighed, and individually placed into wells filled with 6 mL of the respective buffer solution. All six conditions (pH 3, 7, 11, and pH 7 at 10, 50, and 100 mM NaCl) were tested in triplicate samples. Similarly, three aa-HEC/4h-EDTA pucks at the optimal a:h ratio were also made as a control (no aa-CMC) comparison (in pH 7 phosphate buffer with 0 mM NaCl). The pucks were weighed and photographed over six weeks to track the evolution of their mass (g) and diameter (cm), respectively. The images were taken by placing the six well plates above a transilluminator on a TL-2000 Ultraviolet Translinker (Ultra Violet Products, Haverhill, Massachusetts, USA). The area was measured using ImageJ software (NIH, Bethesda, MD) and used to calculate the normalized diameter (cm³, assuming isotropic expansion) for direct comparison with the normalized mass (g/g) (**Appendix A, Figure A9A-B**).

2.2.8 Sorption Isotherms

To study how the sorbate (Cu^{2+}) interacts with the sorbent (aa-HEC/aa-CMC/4h-EDTA hydrogel), sorption isotherm data was collected at room temperature and pH = 5-6 in triplicate at initial Cu²⁺ concentrations of 40 to 800 mg Cu^{2+/} g hydrogel. More specifically, hydrogel monoliths were prepared as described earlier, and 0.25 mL of a 100 mM NaCl/10 mM acetate buffer solution at pH 5 was added, followed 2 h later by the addition of 0.25 mL of various [Cu²⁺] solutions (480 to 9600 ppm). After 24 h of hydrogel incubation in the NaCl/acetate buffer/Cu²⁺ mixture, all supernatants were removed from the monoliths in the microcentrifuge tubes and placed in fresh 2 mL tubes. After centrifuging for 10 min at 12,000g an aliquot of the supernatant was diluted 50-fold with the addition of nitric acid to 0.2 wt% and water. Calibration standards of Cu²⁺ ranging from 0 to 100 ppm were prepared in an aqueous solution containing 0.2 wt% HNO₃, 1 mM NaCl and 0.1 mM acetate buffer. All samples and calibration standards were analyzed using the Vista-PRO CCD Simultaneous ICP-OES (Varian, Mississauga, ON, Canada). Emission intensity at 213.598 nm for Cu²⁺ was recorded by Varian's ICP Expert II system software, and a calibration curve (Appendix A, Figure A10A) was plotted using corrected emission intensity. A linear regression was used to determine the Cu²⁺ concentrations in the supernatant samples. In all experiments, the Cu^{2+} sorption capacity at equilibrium (q_e) was calculated using (Eq. 2.1):

$$q_e = \frac{(C_i V_i - C_e V_T)}{m} \tag{2.1}$$

where $q_e (\text{mg} \cdot \text{g}^{-1})$ is the sorption capacity at equilibrium; C_i and C_e are the initial and equilibrium copper concentration (ppm); V_i and V_T is the initial and total volume of the solution; and *m* is the dry mass (g) of the sorbent. The average $q_e (\text{mg} \cdot \text{g}^{-1})$ was plotted as a function of average C_e (ppm) to obtain the hydrogel sorption isotherm, which was modelled using two parameter non-linear isotherm models – Langmuir (Eq. 2.2) and Freundlich (Eq. 2.3), and a three-parameter non-linear isotherm model – Langmuir- Freundlich (Eq. 2.4):

$$q_e = \frac{q_{max} \cdot K_L \cdot C_e}{1 + K_L \cdot C_e}$$
(2.2)

$$q_e = K_F \cdot C_e^{1/n} \tag{2.3}$$

$$q_e = \frac{q_{max} \cdot K_{LF} \cdot C_e^{1/n}}{1 + K_{LF} \cdot C_e^{1/n}}$$
(2.4)

where $q_e (\text{mg} \cdot \text{g}^{-1})$ is the sorption capacity at equilibrium; $C_e (\text{ppm})$ is the equilibrium concentration of aqueous copper; $q_{max} (\text{mg} \cdot \text{g}^{-1})$ is the theoretical maximum sorption capacity; and the following are all experimentally determined constants: $K_L (L \cdot \text{mg}^{-1})$ is the Langmuir constant, K_F is the Freundlich constant, K_{LF} is the Langmuir-Freundlich affinity constant, and *n* is the Freundlich exponent.

2.2.9 Sorption Kinetics

To assess the kinetics of Cu²⁺ sorption to the aa-HEC/aa-CMC/4h-EDTA hydrogel monoliths, a kinetic study was performed in triplicate measuring supernatant concentrations at 12 time points ranging from 0 to 24 h. Hydrogel monoliths were prepared as described earlier, and 0.25 mL of a 100 mM NaCl/10 mM acetate buffer solution at pH 5 was added, followed 2 h later by the addition of 0.25 mL 7200 ppm Cu²⁺ solution. The supernatant was removed in 5 μ L aliquots at the selected timepoints, which were diluted 100-fold with water and centrifuged for 10 min at 12,000g. Then an aliquot of the supernatant was diluted two-fold with the addition of PEI to 0.05 wt% and analyzed for the [Cu²⁺] using the PEI-Cu binding assay as described later. The hydrogel binding kinetics data was fitted with the intraparticle diffusion (IPD) model (**Eq. 2.5**). Using the information obtained from the IPD model, a portion of the kinetics data was fitted to both the non-linear pseudo-first order (**Eq. 2.6**) and non-linear pseudo-second order (**Eq. 2.7**) kinetic models:

$$q_t = k_{id} \cdot t^{0.5} + C \tag{2.5}$$

$$q_t = q_e \left(1 - e^{-k_1 \cdot t} \right) \tag{2.6}$$

$$q_t = \frac{q_e^{2} \cdot k_2 \cdot t}{1 + k_2 \cdot q_e \cdot t}$$
(2.7)

where q_t (mg·g⁻¹) and q_e (mg·g⁻¹) are the sorption capacity at time *t* and at equilibrium, respectively. The k_{id} (mg·g⁻¹·min^{-0.5}) is the rate constant of the IPD model, and *C* (mg·g⁻¹) is a constant involved in the thickness of the boundary layer of the IPD model. The k_l (min⁻¹) and k_2 (g·mg⁻¹·min⁻¹) are the pseudo first and second order equilibrium rate constants, respectively. It is important to note that for the kinetics study, the q_l is calculated similarly to the sorption capacity noted above in **Eq. 2.1**, but, the sorbate supernatant concentration at each time point (C_l) is used, and the total volume is corrected by taking into account both the volume reduction from the 5 µL aliquot removals at each time point ($V_{T,l}$) and the cumulative removed sorbate mass ($\sum_{i=1}^{t} C_t V_r$), where volume removed (V_r) is 5 µL (**Eq. 2.8**).

$$q_t = \frac{(C_i V_i - C_t V_{T,t} - (\sum_{0}^{t} C_t V_r))}{m}$$
(2.8)

2.2.10 Copper Quantification through the PEI-Cu Binding Assay

A sensitive, rapid and simple spectrophotometric method for Cu^{2+} detection at various time points of the kinetics study was implemented using PEI, a colorless polymer which turns bright blue upon complexation with $Cu^{2+,25}$ Cross-validation of this method with the gold standard inductively coupled plasma optical emission spectroscopy (ICP-OES) showed excellent agreement (**Table A1**). Absorption spectra of PEI-Cu samples recorded by Tecan i-Control software using an Infinite 200 plate reader found maximum absorbance at $\lambda_{max} = 273$ nm (**Appendix A, Figure A11A**). Calibration standards of PEI-Cu ranging from 0 to 50 ppm Cu²⁺ were prepared with a 0.05 wt% PEI, 0.25 mM NaCl and 0.025 mM acetate buffer matrix. Calibration standards and kinetic samples were pipetted into a Greiner UV-Star 96 well plate (200 μ L), and absorbance at $\lambda_{max} = 273$ nm was recorded. A calibration curve was plotted using corrected absorbance, with 0.05 wt% PEI used as the blank (**Appendix A, Figure A11B**). Linear regression was used to determine the Cu²⁺ concentrations in the supernatant samples, accounting for the 200-fold dilution during analysis.

2.2.11 Multi-metal Sorption Comparison

To study how various heavy metals interact with the hydrogel, sorption capacity data of copper (Cu²⁺), nickel (Ni²⁺), zinc (Zn²⁺), cobalt (Co²⁺) and magnesium (Mg²⁺) ions was collected in triplicate at room temperature and pH = 5-6 using an initial concentration of 600 mg metal ion/g hydrogel. This data was also collected in triplicate for a mixture, where each of the five heavy metals made up an equal portion of the initial concentration. More specifically, hydrogel monoliths were prepared as described earlier, and 0.25 mL of a 100 mM NaCl/10 mM acetate buffer solution at pH 5 was added, followed 2 h later by the addition of 0.25 mL of the respective metal solution at 7200 ppm. After 24 h of hydrogel incubation in the salt/buffer/metal²⁺ mixture, all hydrogel supernatants were removed from the monolith tubes and placed into fresh 2 mL tubes and centrifuged for 10 min at 12,000g. An aliquot of the supernatant was diluted 50-fold with the addition of nitric acid to 0.2 wt% and water. Multi-metal calibration standards of Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺ and Mg²⁺, ranging from 0-100 ppm, were prepared with a 0.2 wt% HNO₃, 1 mM NaCl and 0.1 mM acetate buffer matrix. All samples and calibration standards were analyzed using the Vista-PRO CCD Simultaneous ICP-OES (Varian, Mississauga, ON, Canada). Emission intensity at 213.598, 216.555, 213.857, 230.786 and 285.213 nm for Cu2+, Ni2+, Zn2+, Co2+ and Mg2+, respectively, was recorded by Varian's ICP Expert II system software, and calibration curves (Appendix A, Figure A10) were plotted using corrected emission intensity. Linear regression was

used to determine the metal ion concentrations in the supernatant samples. In all experiments, the metal sorption capacity at equilibrium (q_e) was calculated using Eq 2.1.

2.3 Results & Discussion

2.3.1 Quantification of Aldehyde Content on aa-HEC & aa-CMC

The starting reactive materials aa-HEC, aa-CMC and 4h-EDTA were synthesized in high yields and with high purity, and characterized using NMR and FTIR, with 4h-EDTA and its intermediate also characterized by ESI-MS (see **Figure A1**, **A3**, **A4**, **A5** and **A6** in **Appendix A**). The ¹H NMR spectra of both aa-HEC and aa-CMC confirmed the presence of vanillin, while the ¹H and ¹³C NMR spectra of EDTA tetraethyl ester and 4h-EDTA confirm their synthesis and purity. The FTIR spectrum of aa-HEC indicates the presence of vanillin by the C=C stretching at 1466 cm⁻¹ and carbonyl stretching at 1730 cm⁻¹ (**Figure 2.2A**). The peaks at 1640, 1610 and 1590 cm⁻¹ are characteristic of the C=N stretch from the triazine moiety which is used to graft the vanillin onto the anhydroglucose units. The FTIR spectrum of aa-CMC shows stretching at 2943 and 1774 cm⁻¹, which is ascribed to the aromatic C-H and carbonyl stretching, respectively, from vanillin. The 4h-EDTA crosslinker, shows stretching peaks from 3400-3300 and 3300-3250 cm⁻¹, which are characteristic of the aliphatic primary amines of the hydrazide groups (**Appendix A, Figure A5**).



Figure 2.2. Characterization of cellulose derivatives before and after modification. (A) FTIR spectra of HEC, aa-HEC, CMC and aa-CMC. (B) Representative UV-Vis spectra for vanillin (60 μ M), aa-HEC, aa-CMC, unmodified HEC and CMC (all at 0.05 wt%) used to determine aldehyde content on aa-HEC and aa-CMC.

Beyond confirming the presence of aromatic aldehydes on aa-HEC and aa-CMC through FTIR spectroscopy, we quantified the aromatic aldehyde content on the aa-HEC and aa-CMC polymer chains to enable the optimization of the aldehyde:hydrazide (a:h) crosslinking ratio to form stable hydrogels. Therefore, the aromatic aldehyde degree of functionalization (DoF) on the aa-HEC and aa-CMC was determined using UV-Vis spectrophotometry. The conjugated system in vanillin makes it UV active, which allowed us to compare the absorption spectra of vanillin to that of aa-HEC and aa-CMC (Figure 2.2B). Multiple absorption peaks between 230-420 nm were observed for the modified polymers, while unmodified HEC and CMC showed no absorbance peaks in this range. Specifically, aa-HEC and aa-CMC exhibited absorbance maxima at $\lambda_{max} = 310$ nm, that coincide with vanillin's absorbance, while there was no signal at this wavelength in the unmodified HEC and CMC. Thus, vanillin standards ranging from 0-60 μ M were used to make an absorbance calibration curve (Appendix A, Figure A2) to determine the aldehyde content on the aa-HEC and aa-CMC. The concentration of vanillin was found to be 32 μ M and 11 μ M in 0.05 wt% aa-HEC and aa-CMC samples, respectively. Assuming each cyanuric chloride was modified with a single vanillin molecule, the aa-HEC and aa-CMC had a DoF of 1.6 and 0.5 aromatic aldehydes per 100 anhydroglucose units (AGU), respectively (calculations can be found in the Appendix A). The lower aldehyde content in aa-CMC compared to aa-HEC was attributed to the one-pot reaction being less effective for CMC. This is expected given that only 30% AGU have primary hydroxyl groups available for reaction with the vanillin-triazinyl derivative, while HEC chains have primary hydroxyl groups available on the unmodified and the hydroxyethyl-modified AGUs. Hydrogel properties, such as gelation time and mechanical robustness, were quantitatively tuned (using the aromatic aldehyde DoF information) by changing the aldehyde to hydrazide (a:h) ratios and the polymer concentrations. This was useful to control for variability in DoF between different batches

of aa-HEC and aa-CMC. It is also important to note the aromatic aldehyde content within a batch was observed to decrease by 9% and 17% for aa-HEC and aa-CMC, respectively, after one year of storage at 4 °C, which should be taken into account when using the materials after prolonged storage.

2.3.2 Mechanical Properties

Mixtures of aa-HEC and 4h-EDTA with well-defined a:h ratios were tested to find mixtures that produced robust aa-HEC/4h-EDTA hydrogels in a timeframe of minutes. A range where either the aldehyde or the hydrazide was in excess was also of interest to qualitatively assess the impact on gelation times and mechanical robustness. Figure 2.3A schematically illustrates this by keeping a constant number of aa-HEC chains, each bearing a fixed number of aldehyde functionalities, and progressively adding more 4h-EDTA molecules, each with four hydrazide functionalities. Tube inversion tests were used to find gelation times for 1:1, 1:2 and 1:4 a:h ratios, which resulted in 2.5, 2 and 1.75 minutes, respectively. As hypothesized, the gelation time decreased with increasing hydrazide content since more crosslinking groups leads to faster formation of a crosslinked network. Vial inversion tests gave us a sense of the optimal a:h ratio favoring greater hydrazide content. Another parameter that needed to be determined was the minimum concentration of aa-HEC needed to obtain hydrogels that were robust enough to be handled. Too little aa-HEC would result in weak hydrogels since the crosslinking and entanglement of the aa-HEC chains would be limited. We made pucks with different concentrations of aa-HEC and qualitatively tested their robustness by pressing on them. Using 1.5 wt% aa-HEC resulted in soft pucks that could not be appropriately handled, while 2 wt% aa-HEC gave pucks that were sufficiently robust for experimentation. Overall, the qualitative vial inversion and puck pressing tests led us to choose 2 wt% aa-HEC as the cellulose concentration to move forward with and postulate that hydrogels

made from a range of 4:1 to 1:4 a:h ratios would allow us to optimize the formulation for maximum robustness.



Figure 2.3. Determination of mechanical properties of 2 wt% hydrogels through rheological measurements. (A) Schematic of the hydrogel crosslinking behavior when hydrazide content (blue tips) is increased while keeping the aldehyde content (purple cones) constant. **(B)** Summary of the storage modulus (G') and loss modulus (G") for aa-HEC/4h-EDTA in-situ gelled samples and pre-gelled pucks at various aldehyde-to-hydrazide (a:h) ratios. **(C)** Summary of G' and G" for

pre-gelled 1:2 a:h aa-HEC/aa-CMC/4h-EDTA pucks with various aa-HEC/aa-CMC (H/C) proportions. **(D)** Summary of G' and G'' for pre-gelled 1:2 a:h 25/75 H/C aa-HEC/aa-CMC/4h-EDTA pucks after 24 h of incubation in various solutions (hydrogels after crosslinking – no incubation control, or after incubation with 10 mM pH 7 phosphate buffer with 0 mM NaCl; 10 mM pH 7 phosphate buffer with 100 mM NaCl; or excess Cu^{2+} solution – 208 mg Cu^{2+} /g). Note: all reported values are averages and standard deviations of measurements performed in n = 3 independently prepared replicate samples.

Rheological tests were performed to quantitatively determine the ratio of a:h that resulted in the most robust 2 wt% hydrogels. **Figure 2.3B** shows G' and G" for the aa-HEC/4h-EDTA hydrogels with different a:h ratios. The similarity between the G' and G" of the pre-gelled pucks and in-situ gelled materials indicates that the materials gel quickly and yield similar mechanical properties as the 24 h pre-gelled pucks after only 10 minutes incubation on the rheometer. A gelation timeframe of minutes is attractive because it allows sufficient time to mix and transfer the materials into molds, while also achieving complete crosslinking/gelation in short incubation times. The slower crosslinking kinetics is possible thanks to the aromatic aldehyde group, which reacts with hydrazides more slowly than the aliphatic aldehyde group.^{26,27} In-situ gelled samples and pregelled pucks showed an increase in the G' and G" as the hydrazide content increased, up to a maximum seen for the 1:2 a:h ratio. Increasing the hydrazide content beyond 1:2 a:h resulted in a concomitant decrease of G' and G".

The trends observed in the storage and loss moduli can be explained through a model that details the formation of a crosslinked network of aa-HEC polymers by a tetra-functional 4h-EDTA crosslinker. Too few hydrazide groups (4:1 a:h ratio) limit the crosslinking and the hydrogel is largely held together via cellulose polymer entanglement and other non-covalent interactions. As the number of 4h-EDTA molecules increases, the crosslink density increases, forming a reticulated network. The hydrogel network is most robust when the greatest number of glucan chains can be covalently crosslinked. In the case of the aa-HEC/4h-EDTA system, this occurs at the 1:2 a:h ratio, where the hydrogel showed the highest G' (~1080 Pa). While one might expect that a 1:1 correspondence in aldehyde-to-hydrazide functionality would be the ideal mixture to obtain the strongest hydrogels, this was not the case in practice due to the multivalency of the 4h-EDTA and the fact that the cellulose polymer chains may not be able to arrange themselves to have optimal crosslinking. Increasing the ratio beyond 1:2 resulted in a weakening of the hydrogels because too many hydrazide groups prevent crosslinking between cellulose chains. At higher ratios, the aldehyde groups will be saturated with hydrazide functionalities from excess 4h-EDTA molecules, forming dangling ends instead of crosslinks between chains, as depicted in **Figure 2.3A**. In summary, the 1:2 a:h ratio was found to be the ideal mixture due to its mechanical robustness.

While the aa-HEC/4h-EDTA system allowed easily tuning the robustness, aa-HEC does not bind heavy metals efficiently and the contribution to metal binding from the crosslinker is small (~2.58 mg/g from a 2 wt% 1:2 a:h aa-HEC/4h-EDTA hydrogel, where each 4h-EDTA binds a single Cu²⁺ ion). Thus, incorporation of aa-CMC is needed to introduce carboxylate groups into the hydrogel system that result in high metal-binding capacity. On the other hand, maintaining a fraction of aa-HEC is necessary to enhance the mechanical robustness, thanks to the three-fold greater aldehyde content and absence of electrostatic repulsion on the polymer backbone. To determine the maximum amount of aa-CMC we could incorporate into the hydrogel without compromising robustness, the optimal crosslink ratio of 1:2 a:h was used to test proportions of aa-HEC to aa-CMC (H/C), from 100/0 to 0/100, while keeping the total cellulose content at 2 wt%. Frequency sweeps and data analysis to determine the G' and G" were performed on pre-gelled aa-HEC/aaCMC/4h-EDTA pucks (**Figure 2.3C**). The trends observed in the storage and loss moduli can be explained by the formation of a crosslinked network of aa-HEC/aa-CMC polymers with a 1:2 a:h ratio. As the proportion of aa-CMC is increased, the crosslink density decreases since aa-CMC has lower aldehyde content, forming a weaker hydrogel network. Thus, the hydrogel is largely held together via physical entanglement and other non-covalent interactions. We selected the 1:2 a:h ratio with a 25/75 H/C composition as the optimal formulation to perform all subsequent experiments, since this aa-HEC/aa-CMC/4h-EDTA hydrogel showed a G' of ~200 Pa and could be easily handled.

The impact of ionic strength on the mechanical properties and stability of aa-HEC/aa-CMC/4h-EDTA hydrogel pucks (2wt%, 1:2 a:h, 25/75 H/C) was tested through rheological measurements after being incubated in different solutions for 24 h. Pucks incubated in 10 mM pH 7 phosphate buffer supplemented with 0 or 100 mM NaCl showed a slight decrease in G' from 198 Pa, for the as-prepared control puck, to 113 and 106 Pa, respectively (Figure 2.3D). This drop in robustness was anticipated due to the swelling of the hydrogel in pH 7 solutions where the carboxylate groups of CMC are negatively charged. The increased ionic strength did not completely abolish the swelling of the hydrogel, and minimally impacted the hydrogel's mechanical strength compared to the salt free pH 7 conditions. The structural stability at 0 and 100 mM NaCl indicates the hydrogel crosslinks are sufficient to prevent the hydrogel from breaking apart despite having increased swelling in the 0 mM NaCl phosphate buffer solution, but that they are not enough to prevent swelling due to electrostatic repulsion as described below (Figure 2.4A). Incubating the hydrogel in an excess Cu^{2+} solution (208 mg Cu^{2+}/g hydrogel) showed a ~15-fold increase in the storage modulus to ~3 kPa, over the control puck. Crosslinking via ionotropic gelation (aka polyelectrolyte complexation)²⁸ from the interaction of Cu²⁺ with the anionic aa-CMC matrix is a beneficial outcome for the application of heavy metal remediation since the hydrogel becomes more robust and causes water to be expelled from the hydrogel when excess metal is present. Although the 2 wt%, 1:2 a:h, 25/75 H/C aa-HEC/aa-CMC/4h-EDTA hydrogel with a G' of ~198 Pa is not very robust, the increase in robustness upon metal binding makes it a reliable single-use, cost-effective, and biodegradable sorbent for heavy metal remediation. This hydrogel platform offers a sorbent format that can be easily removed after heavy metal sorption unlike most industrial sorbents which are powders that lead to poor sorbent removal efficiency and secondary pollution.

2.3.3 Hydrogel pH Stability

pH stability is another major consideration for the application of hydrogels to heavy metal remediation of polluted water. The aa-HEC/aa-CMC/4h-EDTA hydrogels are held together via hydrazone bonds, which are known to be stable in the 5-9 pH range.²⁹ We evaluated the stability of the hydrogels in the 3-11 pH range to determine their potential for heavy metal remediation, given that EDTA and carboxylates present high affinity for metal ions at high pH and reduced affinity at low pH. This property could be useful for metal chelation and subsequent release by changing the pH in which the hydrogels are immersed. The stability of the optimal aa-HEC/aa-CMC/4h-EDTA hydrogel system (2 wt%, 1:2 a:h, 25/75 H/C) was tested over a six-week period by incubating gelled pucks in solutions at pH 3, 7, and 11 and at pH 7 with increasing salt content (0 to 100 mM NaCl). The stability was evaluated qualitatively via photographs (Figure 2.4A) and quantitatively by measuring and plotting the normalized hydrogel diameter (Appendix A, Figure A9A-B) and normalized hydrogel volume (Figure 2.4B-C). The puck masses were also recorded, and the evolution of the normalized hydrogel mass was plotted over time (Appendix A, Figure A9C-D). This additional measure helped us to better understand the hydrogel's temporal degradation, since image analysis alone could only evidence swelling and surface degradation,

while tracking mass accounted for swelling and bulk erosion. Bulk erosion could lead to negligible changes in diameter even if the material were degrading, but changes in hydrogel mass would be evidence of bulk erosion.³⁰



Figure 2.4. Hydrogel stability over six-week incubation in various environments. (A) A series of 2 wt%, 1:2 a:h, 25/75 H/C aa-HEC/aa-CMC/4h-EDTA pucks were incubated in 10 mM buffer solutions with 0.02 wt% NaN₃ at pH 3, 7 and 11, and pH 7 phosphate buffer with 0, 10, 50 and 100 mM NaCl; and 2 wt%, 1:2 a:h, aa-HEC/4h-EDTA pucks (no CMC hydrogel control) in pH 7 phosphate buffer with 0 mM NaCl imaged over 42 days (~1000 h). Temporal evolution of the normalized hydrogel volume in environments with different (B) pH and (C) ionic strengths. Note: All error bars represent a standard deviation of n = 3 independently prepared replicate pucks.

Under acidic conditions, the pucks showed lower swelling than in neutral and basic conditions over the first 10 h. This was expected since at pH 7 and 11 the carboxylic acid groups are deprotonated (pKa = 4-5) and the negatively charged carboxylate groups cause the hydrogel to swell through electrostatic repulsion and water uptake, while no such repulsion was expected at pH 3. However, after the 24 h point the swelling in pH 3 solutions was greater than in pH 7 solutions, and surpassed both pH 7 and 11 solutions by the 7-day time point (168 h), as tracked through photographs and the normalized volume. This trend continued until 21 days (504 h), after which the hydrogel at pH 3 was not visually traceable due to complete degradation. Furthermore, the mass of the hydrogel pucks in acidic conditions (Appendix A, Figure A9C) remained lower than in the basic and salt-free neutral conditions despite having a similar normalized volume at 24 h, indicating the bonds were not being broken by osmotic pressure/ water-uptake, but rather the hydrazone crosslinks were being hydrolyzed in the acidic pH. The mass was not recorded past the 48-h mark for the pucks in acidic conditions since they were too soft to handle (remove and weigh) without breaking. Given that the cellulose-based hydrogel is inexpensive, when compared to synthetic sorbents, valuable but toxic metals bound to the cellulose hydrogels could be recovered by degrading the hydrogels under acidic conditions. This would allow for quick resource recovery

while avoiding the time-consuming regeneration and washing steps which lack efficiency in most high binding materials due to lengthy diffusion times.

In pH 11 and pH 7 conditions (Figure 2.4B), and neutral conditions with increasing ionic strength (Figure 2.4C), there was an increase in the normalized hydrogel volume until 24 h followed by a noticeable drop (24 h - 7 days) and rise (7 days - 42 days), while the normalized masses plateaued and started a shallow decline beyond 24 h (Appendix A, Figure A9C-D). We expected the normalized volume and mass to follow similar trends since volume and mass should change in lock step, as was indeed seen for the swelling over the first 24 hours. However, the trends did not agree after 24 h, where it was observed that the puck volume decreased and then experienced a second expansion, while the mass steadily decreased. We postulated that the second expansion and steady loss of mass could be due to the crosslinking bonds degrading overtime. Materials crosslinked through hydrazone bonds are in dynamic equilibrium at pH 7, meaning that they can constantly break and reform. We hypothesized that the electrostatic repulsion between the aa-CMC polymers caused them to drift apart and prevented hydrazone bonds that broke from reforming. This would lead to hydrogel swelling from a progressive decrease in bond density and a concomitant loss of mass, as untethered polymer chains escaped from the hydrogel monolith. This hypothesis was supported by the observation that increasing the ionic strength at pH 7 stabilized the hydrogels. While the second swelling was still observed, increasing the ionic strength significantly reduced this effect, and the mass remained constant over six weeks; screening the charge of the carboxylate groups slowed down hydrogel degradation.

To confirm that the charged carboxylate groups were the origin of the mismatch between the volume and mass at timepoints after one week, a control puck made from 1:2 a:h, aa-HEC/4h-EDTA was tracked in pH 7 phosphate buffer with 0 mM NaCl over six weeks. The volume of the

aa-HEC pucks was effectively stable past the 24 h timepoint and did not exhibit the drop and rise trend observed for pucks containing aa-CMC. The mass was also stable past the 24 h timepoint. In general, the aa-HEC control hydrogel showed no change in its volume or mass over six weeks as normalized values remained close to one. Observing the control hydrogel pucks, which lacked aa-CMC, leads us to conclude that the aa-HEC/aa-CMC/4h-EDTA hydrogel system (2 wt%, 1:2 a:h, 25/75 H/C), degrades overtime via hydrazone bond breaking and bulk erosion due to repulsion by the carboxylate groups in CMC chains. Overall, these results indicate that the aa-HEC/aa-CMC/4h-EDTA hydrogels are robust and stable in neutral and basic environments for one week and begin to degrade slowly after that point. Furthermore, higher ionic strength solutions help in stabilizing the hydrogels via charge screening and slow down the swelling and progressive degradation.

2.3.4 Sorption Isotherms

Prior to incubation with heavy metals, aa-HEC/aa-CMC/4h-EDTA hydrogel monoliths of the optimal composition (2 wt%, 1:2 a:h, 25/75 H/C) were pre-conditioned in 0.25 mL salt/ buffer solution (100 mM NaCl/10 mM acetate buffer at pH 5) for 2 h. This maintained the pH in the 5-6 range, which is high enough to deprotonate the carboxylate groups on the cellulose,³¹ while avoiding metal precipitation which is known to occur at higher pH.¹⁶ Then 0.25 mL Cu²⁺ solutions (480 to 9600 ppm) were added to assess the sorption capacity of the hydrogel and to understand the interaction between the sorbent (hydrogel) and sorbate (Cu²⁺) at equilibrium. The hydrogel sorption isotherm was modelled using the two-parameter non-linear isotherm Langmuir and Freundlich models, and the three-parameter non-linear isotherm Langmuir-Freundlich model (**Figure 2.5A**). These models provided crucial insights into the nature of the sorption process, such

as whether sorption was due to physical forces (physisorption) or chemical bonds (chemisorption), the heterogeneity of the surface (varying binding affinities), and maximum sorption capacities.



Figure 2.5. Sorption studies of 2 wt%, 1:2 a:h, 25/75 H/C aa-HEC/aa-CMC/4h-EDTA hydrogel monoliths. (A) Sorption Isotherms. Non-linear fitting of the hydrogel binding isotherm ($C_0 = 240-4800$ ppm in 50 mM NaCl and 5 mM acetate buffer, t = 24 h, T = 298 K, V = 0.65 mL) using the Langmuir, Freundlich and Langmuir-Freundlich models, where n = 3 independent samples for each point. Inset: pictures of the hydrogel monoliths after removing the supernatant at 24 h. Sorption Kinetics of Cu²⁺ ($C_0 = 3600$ ppm in 50 mM NaCl and 5 mM acetate buffer, T = 298 K, V = 0.65 mL) tracked over 24 h and fitted to the (**B**) intraparticle diffusion model, and (**C**)

non-linear pseudo-first-order (PFO) and non-linear pseudo-second-order (PSO) kinetic models (data fitted for 2 h onwards), where n = 3 independent samples used for each point. **(D) Multi-metal Sorption Comparison.** Equilibrium sorption capacities of hydrogels for metal ions (Cu²⁺, Zn²⁺, Co²⁺, Ni²⁺ and Mg²⁺) tested individually, or together as a multiple-component system over a period of 24 h (C₀ = 3600 ppm in 50 mM NaCl and 5 mM acetate buffer, T = 298 K, V = 0.65 mL), where n = 3 independent samples for each metal condition.

The parameters obtained from fitting each isotherm model are summarized in **Table 2.1**. Fitting to the Langmuir model, the 2 wt%, 1:2 a:h, 25/75 H/C aa-HEC/aa-CMC/4h-EDTA hydrogel monolith achieved a maximum binding capacity of 102 mg of Cu^{2+} per g of dry hydrogel. Looking at the Freundlich exponent n (heterogeneity factor), values between 0-1 imply weak adsorptive forces on the sorbent's surface, while values above one denote beneficial adsorption, where the magnitude of *n* depicts the adsorption intensity.³² Although Cu^{2+} shows beneficial adsorption to the hydrogel monolith in the Freundlich model, the Langmuir model more accurately describes the experimental data. The Langmuir isotherm model assumes a homogenous sorbent surface where all binding sites have the same binding energy and indicates that the Cu²⁺sorbate is binding in a single layer with equal binding energies at all sites, likely through chemisorption (i.e., coordination of the metal by the carboxylate groups on the aa-CMC. Lastly, the three-parameter non-linear Langmuir-Freundlich isotherm model presented the best fit ($R^2 = 0.98$), indicating a combined behavior ascribed to variation in the affinity of the binding sites, such as Cu²⁺ ions interacting with 4h-EDTA groups (coordination via free amine groups) and physisorption (from multi-layer binding, especially present at the hydrogel monolith surface due to Cu²⁺ ions overcoming mass transfer resistance at the boundary layer). Interestingly, all models fitted poorly in the lower concentration range where the hydrogel monoliths were not able to bind the Cu²⁺ present in the

aqueous phase despite having an abundance of binding sites and exhibiting swelling. The lack of binding at the lower sorbate concentrations is likely due to the concentration gradient not being high enough to overcome the diffusion hindrance present at the boundary layer (discussed below). This effect is likely exacerbated by the surface becoming crosslinked from the initial binding of Cu^{2+} , causing crosslinking via polyelectrolyte complexation, and thus shrinking entry sites/pores. This way at low sorbate concentrations, only water, which is neutral, can enter the hydrogel monolith due to osmosis, yet the remaining Cu^{2+} cannot diffuse past the initial binding sites due to mass transfer resistance from the crosslinked surface.

Isotherms	Parameters			
	$q_{\max} (mg \cdot g^{-1})$	$K((L \cdot g^{-1})^{1/n})$	n	R ²
Langmuir	102.23	6.01	-	0.94
Freundlich	-	13564.39	3.91	0.82
Langmuir-Freundlich	90.71	0.012	0.43	0.98

 Table 2.1. Parameters of the non-linear sorption isotherms.

Based on the model fits, we note that although this hydrogel is within a competitive range of sorption capacity compared to other cellulose-based sorbents,^{21,33} a limitation is that the Langmuir constant K_L , representing the affinity between the sorbate (Cu²⁺) and sorbent (hydrogel) is lower than desired. A future goal should be to improve K_L , which would result in a sorbent with a higher binding affinity. We also observed that this sorbent system is most efficient at high sorbate concentrations, which provide an osmotic driving force for the ions to enter the hydrogel and overcome the mass transfer resistance between the liquid and gel phase.³³ We found that initial Cu²⁺ concentrations above 200 mg•g⁻¹ provided enough osmotic driving force for the hydrogels to

bind metal ions efficiently. This effect can be observed qualitatively as the hydrogel pucks begin to expel water (shrinking), and quantitatively by measuring the expelled NaCl (from the salt/acetate buffer pre-incubation step) by ICPOES (**Appendix A, Figure A12**). With initial Cu²⁺ concentrations above the threshold, the ion-exchange results in almost complete recovery of the initial added Na⁺ ions from the salt/acetate buffer pre-incubation step and no additional changes in the water content of the hydrogel. From these observations, an initial Cu²⁺ concentration that overcomes the mass transfer resistance ($C_i = 600 \text{ mg} \cdot \text{g}^{-1}$) was chosen for the sorption kinetic studies. Overall, the swelling behaviour at low initial sorbate concentrations and shrinking behaviour at high initial sorbate concentrations, along with the good fit to the Langmuir isotherm model, suggests chemisorption as the primary sorption mechanism (via the carboxylates coordinating to Cu²⁺ ions), achieving a maximum binding capacity of 102 mg of Cu²⁺ per g of dry hydrogel.

2.3.5 Sorption Kinetics

In addition to understanding the interaction between the sorbent and sorbate, understanding the stages and rates of sorption is crucial for the practical application of the sorbent. Models used to describe sorption kinetics include the intra-particle diffusion (IPD), pseudo-first-order and pseudo-second-order kinetic models. The IPD model looks at three key stages of sorption when considering solid-liquid systems: (1) boundary layer diffusion (external mass transfer), (2) intra-particle diffusion, and (3) sorption-desorption equilibrium at the binding site, and provides insight into which stage is rate limiting. In **Figure 2.5B**, the plot of q_t versus $t^{0.5}$ yielded three linear regions, which corresponded to the three stages of Cu^{2+} diffusion: (1) external diffusion from the bulk solution to the surface of the hydrogel, (2) diffusion from the surface to the inner pores, and (3) slow diffusion to reach equilibrium. The slopes of the three linear regions correspond to the

diffusion rate k_{id} (mg·g⁻¹·min^{-0.5}), and the intercept C (mg·g⁻¹) indicates the thickness of the boundary layer. The constants calculated from the IPD model are summarized in **Table 2.2**. The magnitude of the rate constants decreases progressing from stage one to three, suggesting the rate of sorption at the first stage was very fast, compared to the second and third stages due to the number of available binding sites. Since the rate constant of the initial stage was fastest, the majority of the Cu²⁺ ions diffused from the bulk solution to the hydrogel monolith surface in the first 120 min of diffusion. As the external hydrogel surface became saturated, the Cu²⁺ ions entered the pores, characterized by the second stage lasting 120-360 min. The slower rate of diffusion in the second stage demonstrates increased diffusion resistance, which suggests increased hydrogel crosslinking via polyelectrolyte complexation by the Cu²⁺ as it enters the pores.

The final phase presented a much slower rate of diffusion to the internal surface binding sites, which eventually reached a state of equilibrium after 360-540 min. Additionally, the IPD model revealed that the linear region of stage one did not intersect the origin (i.e. $C \neq 0$). Based on the data obtained through the IPD model, it can be deduced that intraparticle diffusion is not the sole rate-determining step in the Cu²⁺ sorption process; rather the rate of sorption is controlled by both boundary layer and intraparticle diffusion. Since the boundary layer constant *C* is a large negative value for stage one, sorption was complicated by mass transfer resistance from surface binding and a larger boundary layer effect,³⁴ causing a time lag for the sorption process, a phenomenon previously observed in other hydrogel sorbent systems.^{21,32,35} The observed stages illustrate that the overall rate of sorption is not only dependent on the chemical affinity between the sorbate and sorbent but is also significantly influenced by mass transfer processes both external and internal to the sorbent. Based on these results, we suggest that future work should investigate hydrogel

design changes to introduce higher surface to volume ratios that speed up the rate of sorption at the first stage of boundary layer diffusion.

Kinetics Models	Parameters			
—	k_{id} (mg·g ⁻¹ ·min ^{-0.5})	$C (\mathrm{mg} \cdot \mathrm{g}^{-1})$	R ²	
Intraparticle diffusion*	k _{id.1} 21.279	-184.53	0.99	
	<i>k</i> _{<i>id</i>.2} 5.696	-4.429	0.98	
	<i>k</i> _{<i>id</i>.3} 0.631	93.247	0.39	
Pseudo-first-order	0.005727 (min ⁻¹)		0.96	
	0.00005418			
Pseudo-second-order	$(g \cdot mg^{-1} \cdot min^{-1})$		0.92	

Table 2.2. Parameters of the kinetic models summarized.

*Intraparticle diffusion model stages: (I) 0-120 min, (II) 120-360 min, (III) 360-1440 min

As Cu²⁺ binds to the outer surface of the hydrogel, a crust layer is formed with reduced pore size that results in increased mass transfer resistance. Therefore, the initial binding capacity appears as a negative value that gradually becomes positive as Cu²⁺ continues to bind to sites deeper within the hydrogel monolith and starts to plateau at 360 min (during the sorption-desorption equilibrium stage). Given that the hydrogel monolith in the batch sorption system experiences mass transfer resistance from both the boundary layer and the "crust layer", when fitting the data to non-linear pseudo-first order and pseudo-second-order kinetic models, only the data from 120 min onwards was fitted (**Figure 2.5C**). The fitting parameter values and the correlation coefficients are presented in **Table 2.2**. The non-linear pseudo-first-order kinetic fit shows a slightly better R², suggesting physisorption as the sorption mechanism. However, this is impacted by the hydrogel experiencing mass transfer resistance at the boundary layer, and the system is in reality undergoing chemisorption, as seen from the binding isotherm data previously. To address this limitation, future work should focus on reducing the size of the hydrogel monoliths or increase the macroporosity of the system to enhance binding. This would also effectively decrease the time to equilibrium, which is currently around 360-540 min.

2.3.6 Multi-metal Sorption Comparison

In addition to heavy metals leaching from waste sites, heavy metal pollution of water systems originates from mining and industrial processes and includes the simultaneous presence of several different metals. Therefore, the sorption capacity of the hydrogel for various heavy metals (Cu²⁺, Ni^{2+} , Zn^{2+} , Co^{2+} and Mg^{2+}) was tested both individually and as a mixture (Figure 2.5D). Here, the binding capacity was recorded as mmol of metal bound per gram of dry hydrogel mass to allow establishing which metal had the greatest binding affinity. Of all the metals tested individually, Cu^{2+} showed the largest sorption capacity, followed by Zn^{2+} , Co^{2+} , Ni^{2+} and Mg^{2+} (1.21 ± 0.08, $1.13 \pm 0.06, 0.68 \pm 0.03, 0.62 \pm 0.14$ and 0.56 ± 0.15 mmol·g⁻¹, respectively). In the heavy metal mixture, Cu²⁺ had the greatest binding capacity followed by Mg²⁺, Zn²⁺, Co²⁺ and Ni²⁺ (0.79 \pm $0.02, 0.19 \pm 0.05, 0.13 \pm 0.01, 0.09 \pm 0.01$ and 0.06 ± 0.02 mmol·g⁻¹, respectively). The hydrogel selectivity for Cu²⁺ is expected given that carboxylates are hard groups and Cu²⁺ is a borderline element that forms coordination complexes with carboxylates. Additionally, the combined multimetal binding capacity $(1.24 \pm 0.06 \text{ mmol} \cdot \text{g}^{-1})$, although insignificantly greater compared to the sorption capacity for Cu²⁺ alone, suggests the hydrogel has additional binding affinity, likely from alternative binding sites, such as the relatively soft amine lone pairs in the 4h-EDTA serving as potential coordinating sites for heavy metal ions other than copper.

2.4 Conclusion

With increasing heavy metal pollution in our water systems, hydrogels offer a feasible and lowcost solution as remediation sorbents, due to their porous structure, high water absorbency, abundant functional groups, and relatively low crystallinity.³⁶ The goal of this work was to make hydrogels using bio-renewable, biodegradable, and cost-effective materials to efficiently bind heavy metals. Two derivatives of cellulose were functionalized with aromatic aldehydes (aa-HEC and aa-CMC), and an ethylenediaminetetraacetic acid (EDTA)-based crosslinker modified with four hydrazide groups (4h-EDTA) was used to form hydrogels. Rheological tests performed on 2 wt% aa-HEC/4h-EDTA hydrogels showed that the 1:2 aldehyde:hydrazide (a:h) ratio resulted in the most robust hydrogels, which was then used to optimize the proportion of aa-HEC/aa-CMC (H/C). The optimal aa-HEC/aa-CMC/4h-EDTA hydrogel (1:2 a:h 27/75 H/C) had a G' of ~200 Pa and was stable at pH 7 and 11 for over one month, but showed signs of bulk erosion beginning at the one-week timepoint. Sorption of copper to this hydrogel monolith followed the Langmuir model, which evidenced a maximum sorption capacity of 102 mg of Cu^{2+} per gram of dry hydrogel and reached equilibrium within 6-9 h under static conditions. Sorbing various heavy metals individually and in a multi-metal mixture (Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺ and Mg²⁺), this hydrogel monolith showed the highest affinity for Cu²⁺. Overall, we show that cellulose based hydrogels could be used as a green material for the inexpensive and simple removal of heavy metals from metal-polluted waters.

2.5 Acknowledgements

MKG conducted all the experiments, analyzed the data and wrote the first draft of the manuscript. AF and YS assisted with synthesis methods and JU assisted with rheology measurements. JMM provided research oversight and funding and reviewed all drafts of the

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Chapter 3: Nanocomposite Cellulose Hydrogels as Mechanically Robust Sorbents for Heavy Metal Remediation of Water

Abstract

Water is essential for life, yet water scarcity due to pollution by heavy metals is a growing problem severely impacting resource-limited areas where drinking water is already insufficient. Nanosorbents, such as biochar and activated carbon, are currently being employed in wastewater management. However, drawbacks of these powders include high production cost, low regeneration capacity, and concerns regarding their ecological and human health impacts. To overcome these issues, nanocomposite hydrogels are currently being explored as low-cost and safe nanostructured sorbent alternatives which preserve the crucial physicochemical properties needed to effectively remove heavy metals. Our goal was to develop robust, renewable, biodegradable, and cost-effective nanocomposite hydrogel sorbents able to efficiently bind heavy metals. This was accomplished using hydroxyethyl cellulose (HEC) and carboxymethyl cellulose (CMC) functionalized with aromatic aldehydes (aa-HEC and aa-CMC), and native and modified cellulose nanocrystals (CNCs). The cellulose composites were crosslinked with an ethylenediaminetetraacetic acid (EDTA)-based crosslinker modified with four hydrazide groups (4h-EDTA). The nanocomposite hydrogels were templated by adding either native CNCs, aromatic aldehyde modified-CNCs (aa-CNCs), or carboxylated-CNCs (T-CNCs) into the aa-HEC/aa-CMC mixture in equal ratio to the two cellulose polymers (1:1:1), while the 4h-EDTA crosslinker was added to give a 1:2 aldehyde-to-hydrazide crosslinking ratio. These hydrogels were tested for their swelling and mechanical strength after incubating in various conditions (no incubation, 25/10 and 100/10 mM NaCl/acetate pH 5 buffer, and excess Cu²⁺ solution), and for their sorption capacity in presence of excess Cu^{2+} . The 1:1:1 T-CNC nanocomposite hydrogel showed a storage modulus of 150 Pa pre-incubation and 3100 Pa post-incubation in Cu^{2+} (enabling easy handling before and after use), and exhibited the highest sorption capacity for Cu^{2+} of all the nanocomposite hydrogels at 90 \pm 10 mg/g. Considering both mechanical strength and binding capacity, the 1:1:1 T-CNC nanocomposite hydrogel was deemed the optimal composition for the future development of microbeads for use in a column setup to enable water purification of heavy metals at an industrial scale. This work shows that cellulose-based nanocomposite hydrogels could be used as a robust green alternative to powdered nanosorbents for the safe, cost-effective and sustainable removal of heavy metal pollutants from water.

3.1 Introduction

With increasing anthropogenic activities, polluting heavy metals are a growing global problem due to their persistence in the environment leading to bioaccumulation and toxicity.¹ Sorption is an economical and effective method for heavy metal removal due to its high removal efficiency, ease of use, and flexibility in sorbent design.² Nanomaterials are being explored to produce new types of sorbents that have high specific surface area (SSA) and high chemical activity. Emerging nanosorbents for wastewater treatment applications include carbon-based materials such as activated carbon, carbon nanotubes and graphene,^{3,4} metal-based nanosorbents including various nanometal oxides such as aluminum, magnesium, titanium, zinc and others,² and zeolites, which are hydrated aluminosilicate crystalline materials with uniform pore sizes.⁵ Despite the considerable advantages with their tunable pore sizes, high SSA and accessible binding sites, the utilization of nanomaterials for sorption purposes is accompanied by significant limitations, including safety concerns, ⁶ challenges with processing and handling,^{7,8} and potential secondary pollution.⁹ Additionally, the possibility of agglomeration can affect the efficacy and consistency

of sorbent materials, requiring specialized techniques for their synthesis, stabilization and application.^{10,11} Therefore, while nanosorbents demonstrate high sorption capacities for heavy metals — such as polyvinylpyrrolidone-reduced graphene oxide with a sorption capacity of 1689 mg/g for Cu^{2+} ,¹² and magnetite single walled carbon nanotubes/cobalt sulfide nanohybrid,¹³ sorbing Hg²⁺ at 1666 mg/g — the disadvantages associated with their safety, stability in water, processing difficulties, production cost, and secondary pollution risk need to be addressed for upscaled, accessible and sustainable employment to purify water of heavy metal pollution.¹⁴

Cellulose-based nanocomposite hydrogels are currently being explored as low-cost, biodegradable and safe nanostructured sorbent alternatives which maintain the crucial physicochemical properties needed to effectively remove heavy metals.¹⁵ Hydrogels are threedimensional, hydrophilic, cross-linked synthetic or biopolymer networks capable of absorbing water many times their dry weight.¹⁶ While hydrogels made from synthetic polymers offer superior mechanical strength and competitive sorption capacities, they are derived from non-renewable resources and often rely on hazardous cross-linking agents.^{17,18} To overcome these disadvantages, this work focuses on nanocomposite cellulose hydrogels which leverage the favorable properties of hydrogels for water treatment while minimizing environmental impact. Cellulose is a non-toxic, renewable, biocompatible, and biodegradable biopolymer with a linear semicrystalline structure,¹⁹ composed of D-glucose units linked by β-1,4 glycosidic linkages.^{20,21} Our previous work on cellulose-based hydrogels combined an EDTA-based crosslinker bearing four hydrazide groups (4h-EDTA) and two derivatives of cellulose - carboxymethyl cellulose (CMC) and hydroxyethyl cellulose (HEC) functionalized with aromatic aldehyde groups (aa-HEC and aa-CMC). However, these hydrogels are too fragile to be deployable at an industrial scale, which could involve a column packed with hydrogel microbeads.²² Hydrogels need to have both

flexibility and mechanical strength for microbead templating, handling, and dynamic use in a packed column format. Therefore, this work explored the incorporation of native or modified cellulose nanocrystals (CNCs) as green nano-additives in an effort to enhance our previous cellulose-based hydrogel's mechanical strength without compromising its binding capacity. CNCs have served as superior reinforcing materials in the field of nanocomposites and have been incorporated into various polymer matrices due to their unique intrinsic properties such as high SSA, high mechanical strength (elastic modulus of 143 GPa, comparable to Kevlar),²³ and low density.^{19,24} The hydrogel matrix, reinforced with CNCs, not only allows for the effective sorption of heavy metals but also improves the mechanical and physicochemical performance of the material. Examples of CNCs incorporated into the hydrogel polymer matrix for heavy metal sorption include CMC/polyvinyl alcohol/CNC hydrogels (sorption capacity of 109.89 mg/g for Cu^{2+}),²⁵ carboxylated chitosan/carboxylated CNC hydrogel beads (334.92 mg/g for Pb²⁺),²⁶ and corn starch/CNC-based hydrogel (20.3 mg/g for Cu²⁺).²⁷ Overall, the combination of CNCs as bio-renewable, bio-degradable and biocompatible nanoparticles into a polymer matrix compensates for the processing, deployment and retrieval difficulties that powdered nanosorbents face while also enhancing the mechanical strength of the flexible hydrogel matrix.

Since CNCs are colloidally stable in water due to the presence of anionic sulfate half-ester groups on their surface from the isolation step with sulfuric acid hydrolysis,²⁸ the abundance of surface primary hydroxyl groups can be readily modified in water to add crosslinking functionalities (e.g., aromatic aldehydes in our work) or metal chelating functional groups (e.g., carboxylates). Using the same polymer matrix studied in our previous work, where the optimal aa-HEC/aa-CMC/4h-EDTA hydrogel was found to contain a 1:2 aldehyde:hydrazide (a:h) crosslinking ratio, in this work we explored the incorporation of native or modified CNCs at a
1:1:1 aa-HEC/aa-CMC/(modified)-CNC ratio using the 4h-EDTA crosslinker to form dynamic hydrazone bonds at the 1:2 a:h ratio. Pristine CNCs served as a control to assess the mechanical strength enhancement via reinforcing percolated networks. Modified CNCs included aromatic aldehyde modified-CNCs (aa-CNCs) to test for structural strengthening via additional crosslinking sites, and carboxylated CNCs (T-CNCs) to assess for structural reinforcement while introducing carboxylates for metal binding. aa-CNCs were prepared using a similar one-pot synthesis as done for the aa-HEC and aa-CMC, and TEMPO mediated oxidation was used to yield T-CNCs. The nanocomposite hydrogels were compared to previously studied 1:3 aa-HEC/aa-CMC (1:3 H:C) hydrogels by testing the swelling and rheology of pucks incubated in varying ionic strength conditions, and in excess Cu²⁺ incubation. In high ionic strength conditions, all CNC nanocomposite hydrogels showed lower swelling and greater mechanical strength compared to the 1:3 H:C hydrogel. Conversely, incubation in excess Cu²⁺ conditions resulted in all hydrogel compositions experiencing shrinking from polyelectrolyte complexation of Cu²⁺ with the anionic carboxylates in the matrix. The sorption studies using excess Cu²⁺ revealed binding capacities of $110 \pm 6, 90 \pm 10, 70 \pm 20$ and 70 ± 10 mg/g for the 1:3 H:C, 1:1:1 T-CNC, 1:1:1 CNC and 1:1:1 aa-CNC (nanocomposite) hydrogels, respectively. Considering the mechanical strength and binding capacity, the 1:1:1 T-CNC nanocomposite hydrogel — with a storage modulus of 150 Pa pre-incubation and 3100 Pa post-incubation in excess Cu²⁺ — was determined to be the optimal composition for further exploration in bead templating for industrial heavy metal remediation applications. Overall, we showed that CNCs serve as effective green nano-additives, with T-CNCs enhancing both mechanical integrity of cellulose-based hydrogels and providing metal binding sites. Moving forward, these renewable, biodegradable, and mechanically robust nanocomposite cellulose-based hydrogels serve as a basis for further development of sustainable, cost-effective,

and easy to handle (deploy and recover post use) alternatives to powdered nanosorbents for water purification of heavy metals while continuing to implement the principles of green chemistry.

3.2 Experimental Section

3.2.1 Materials

The following chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada) and used without further purification: 2-hydroxyethyl cellulose (HEC, 90,000 Da; lot number: MKCJ9416), sodium carboxymethyl cellulose (CMC, 90,000 Da; lot number: MKCM6314; 0.7 carboxymethyl groups per anhydroglucose unit), vanillin, tetrahydrofuran (THF), cyanuric chloride, acetone, whatman glass filter paper, dialysis membranes (MWCO = 14 kDa), (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO), sodium bromide (NaBr), sodium hypochlorite (NaClO), ethylenediaminetetraacetic acid (EDTA), ethanol (EtOH), sulfuric acid (H₂SO₄), dichloromethane (DCM), sodium bicarbonate (NaHCO₃), 50-60 wt% hydrazine hydrate, sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium chloride (NaCl), sodium acetate (CH₃COONa), acetic acid (CH₃COOH), copper(II) sulfate pentahydrate (CuSO₄·5H₂O), and poly(ethyleneimine) solution (PEI) (average Mn ~60,000 by GPC, average Mw ~750,000 by LS, 50 wt% in H2O). Cellulose nanocrystals (CNCs) were purchased from Celluforce (Montreal, QC, Canada). Phosphate buffered saline (PBS) 10x was purchased from BioShop (Burlington, ON, Canada). Milli-Q water with a resistivity of 18.2 M Ω cm (Milli-Q Advantage A10 Water Purification system, Millipore Sigma, Etobicoke, ON, Canada) was used for all experiments, and is subsequently referred to simply as "water".

NOTE: Refer to Chapter 2 section 2.2.2 for the synthesis of aa-HEC and aa-CMC, section 2.2.3 for the quantification of aldehyde content on aa-HEC and aa-CMC, and section 2.2.4 for the synthesis of 4h-EDTA.

3.2.2 Preparation of Aromatic Aldehyde Modified-CNCs (aa-CNCs)

The chemical grafting of vanillin onto CNCs to obtain aromatic aldehyde modified-CNCs (aa-CNCs) was conducted using a one-pot synthesis procedure. Briefly, 0.5 g of CNCs were left stirring in 50 mL water overnight at room temperature and then probe sonicated with a Sonifier 450 (Branson Ultrasonics, Danbury, CT, USA) in an ice bath for nine 1 min cycles, with 30 s between cycles at 45% amplitude. To the dispersed 1 wt% CNC suspension, 5 mL acetone was added dropwise over 10 min, followed by dropwise addition of 0.0325 g (0.81 mmol) of NaOH dissolved in 2 mL water and 0.051 g (0.34 mmol) vanillin dissolved in 3 mL acetone. After 2.5 h of vigorous stirring at room temperature, 5 mL acetone was added slowly followed by dropwise addition of 0.060 g (0.33 mmol) cyanuric chloride dissolved in 3 mL acetone over a course of 2 min. The reaction was allowed to proceed under vigorous stirring for 1 h prior to purification by dialysis with a membrane (MWCO = 14 kDa) in water for 2 weeks. The dialyzed aa-CNC suspension was centrifuged (10 min at 12,000g) and the supernatant was concentrated to 2 wt% via evaporation before storing at 4 °C in the fridge.

3.2.3 Preparation of Carboxylic Acid Modified-CNCs (T-CNCs)

The protocol by Osorio et al. was adapted to make carboxylated CNCs via (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) oxidation (T-CNCs).²⁹ Briefly, 2.5 g of CNCs were left stirring in 250 mL water at ambient conditions overnight and then probe sonicated with a Sonifier 450 (Branson Ultrasonics, Danbury, CT, USA) in an ice bath for nine 1 min cycles, with 30 s between cycles at 45% amplitude. The dispersed CNC suspension (1 wt%) was poured into a round bottom flask to which a pre-dissolved (stirred at room temperature) 100 mL solution of 0.074 g TEMPO and 0.81 g NaBr was slowly added in 5 mL aliquots. Immediately after, 15 g of 12.5 wt% NaClO was added dropwise; a pH of 10 was maintained using 1 M NaOH. The suspension was

allowed to react for 3 h while pH 10 was maintained, after which 9.1 g EtOH was added and allowed to stir for 10 min to quench the reaction. The T-CNC suspension was dialyzed for two weeks, concentrated to 1 wt% via evaporation, probe sonicated, and vacuum filtered using Whatman glass filter paper before storing at 4 °C in the fridge.

3.2.4 Characterization of Modified-CNCs

To quantify the aldehyde content on aa-CNCs, absorption spectra of vanillin, aa-CNCs and CNCs were recorded by Tecan i-Control software between 230–380 nm using an Infinite 200 plate reader (Tecan, Morrisville, North Carolina, USA) and a Greiner UV-Star 96 well plate (VWR International, Mississauga, ON, Canada) with 200 μ L samples/well, where a λ_{max} of 310 nm was found (**Appendix B, Figure B1**). A vanillin calibration curve (0-60 μ M) was plotted using corrected absorbance values (using water as the blank) at $\lambda_{max} = 310$ nm (**Appendix A, Figure A2**). The modified (aa-CNCs) and control (CNCs) samples were prepared in triplicate as 0.05 wt% solutions and the corrected absorbance at $\lambda_{max} = 310$ nm was used to calculate the degree of functionality of the aa-CNCs.

The carboxylic acid content on the T-CNCs was quantified via conductometric titrations using the MANTECH PRO auto-titrator (Mantech Inc, Guelph, ON, CA) where 1 M HCl and 1 mM NaCl was used to first adjust the pH and conductivity of the T-CNC suspension to ~3 and ~900 μ S/cm, respectively, prior to beginning the auto-titration with 30 μ L aliquots of 0.1 M NaOH solution (LabChem Inc.) added every 30 s. The equivalence volume was determined by subtracting the volumes of the NaOH_(aq) at the two intersections and was used to calculate the moles of carboxylic acid groups on the surface of the T-CNCs (**Appendix B, Figure B2**).

The average particle size of pristine CNCs, aa-CNCs and T-CNCs (0.025 wt % in 10 mM NaCl) was measured by dynamic light scattering (DLS, Malvern Zetasizer 3000, Malvern, UK)

(**Appendix B, Figure B3**). The z-average is the average diameter of a sphere with equivalent Brownian motion to the needle-shaped CNC. To determine colloidal stability of the pristine CNC, aa-CNC and T-CNC dispersions (0.025 wt % in 10 mM NaCl), their zeta potential was measured using a ZetaPlus electrophoretic mobility analyzer (Brookhaven Instruments Corp, Holtsville, NY, USA) (**Appendix B, Figure B4**).

3.2.5 Fabrication of Nanocomposite Hydrogel Pucks & Monoliths

The same protocol used in Chapter 2 section 2.2.5 was used in this work to template nanocomposite hydrogels and obtain 2 wt% aa-HEC/aa-CMC/4h-EDTA hydrogels with a 1:2 aldehyde-to-hydrazide (a:h) crosslinking ratio and 27/75 aa-HEC/aa-CMC (2 wt%, 1:2 a:h, 1:3 H:C). Briefly, to template pucks the 2 wt% stocks of aa-HEC, aa-CMC, and modified-CNCs were pipetted in that order to have a 1:1:1 ratio of the three components, with vortexing for 30 s after each addition. This was followed by the addition of the 4h-EDTA small molecule crosslinker, which was prepared from a buffered stock solution that was diluted to 30 µL with 1x PBS to give a 1:2 a:h ratio upon addition to the respective biopolymers/modified CNCs mixture. The aa-HEC/aa-CMC/modified-CNC/4h-EDTA mixtures were vortexed for 30 s and ~ 180 µL were pipetted into PDMS moulds (diameter = 8.9 mm and height = 3 mm). All pucks were left in the mould for 12 h at room temperature in a sealed hydrated environment before removal for testing their swelling and taking rheological measurements.

The same protocol was followed to make 2 wt%, 1:2 a:h, 1:3 H:C and 1:1:1 aa-HEC/aa-CMC/modified-CNC/4h-EDTA nanocomposite hydrogel monoliths (150 μ L in volume) for metal binding studies. Briefly, the appropriate amounts of each component (with polymers and nanoparticles at 2 wt%) were pipetted, in the order aa-HEC ® aa-CMC ® modified-CNCs ® 4h-EDTA, into a 1.5 mL microcentrifuge tube and vortexed for 30 s after each addition. The

microcentrifuge tubes were capped and left to gel overnight at ambient conditions prior to use. For brevity, the nanocomposite hydrogels are referred to as 1:1:1 CNC, 1:1:1 aa-CNC and 1:1:1 T-CNC, where the 1:3 H:C hydrogel serves as a CNC-free reference and the 1:1:1 CNC as the unmodified/pristine-CNC control to which the aa-CNC and T-CNC are compared.

3.2.6 Swelling & Rheological Tests on (Nanocomposite) Hydrogel Pucks

The robustness of the nanocomposite hydrogels was investigated in various incubation conditions, specifically an incubation-free control of no salt/buffer (no SB), 25 mM NaCl/10 mM acetate buffer pH 5 (25/10 SB), 100 mM NaCl/10 mM acetate buffer pH 5 (100/10 SB), and 2500 ppm Cu²⁺ post 100/10 SB removal (post Cu). In all four conditions (no SB, 25/10 SB, 100/10 SB, post Cu), pucks of four compositions (1:3 H:C, 1:1:1 CNC, 1:1:1 aa-CNC, 1:1:1 T-CNC) were tested in quadruplicate for swelling and subsequent rheological measurements. Briefly, pucks were placed in well-plates and photographed before incubating with 3 mL of the respective salt/buffer (SB) solutions before capping and sealing with parafilm. Note: the no SB control incubation condition involved leaving the pucks in a hydrated environment at ambient conditions. After 24 h, the SB solutions were removed, and the pucks were photographed. Then, using a Discovery Hybrid Rheometer (DTH-20, TA instruments, Mississauga, Ontario, Canada), frequency sweeps were performed on the pucks using an 8 mm geometry at 25°C, with strain of 0.6%, angular frequency of 0.1-100 rad/s and 5 points per decade. Post-rheology, all pucks incubated in the 100/10 SB condition were placed back into their original wells, photographed, and incubated in 2 mL 2500 ppm Cu²⁺ for 1 week, after which excess copper solution was removed, and the post Cu pucks were photographed. Then frequency sweeps were performed on the post Cu pucks using the same settings.

Swelling data analysis involved using the pre- and post-incubation photographs for the 25/10 SB, 100/10 SB and post Cu incubation conditions, where the puck area for all four hydrogel compositions (with four independently prepared replicates pucks each) was measured using the elliptical selection tool in ImageJ software (NIH, Bethesda, MD) and used to calculate the normalized diameter (cm/cm). This was used to calculate the average normalized volume (cm³/cm³, assuming isotropic expansion). For rheology, the storage (G') and loss (G'') moduli were determined for all four hydrogel compositions in all four incubation conditions by taking the average of a fixed range of frequency values (1-10 rad/s) and averaging over the measurements done on four independently prepared pucks. Note: Due to the excessive swelling of the 1:3 H:C pucks incubated in 25/10 and 100/10 SB, they were cut using a 0.8 cm punch prior to doing rheology to maintain size consistency. Therefore, an additional set of 1:3 H:C pucks was incubated in 100/10 SB for 24 h, where the salt/buffer solution was replaced by 2500 ppm Cu^{2+} for one week without cutting the pucks post 100/10 SB incubation. Statistical analysis was performed on the data with the two-tail unpaired t-test used when comparing two independent compositions and the one-way ANOVA used for more than two independent compositions.

3.2.7 Sorption Capacity of (Nanocomposite) Hydrogel Monoliths using Copper

The sorption capacity of the 1:3 H:C hydrogel and the three aa-HEC/aa-CMC/modified-CNC/4h-EDTA nanocomposite hydrogels (1:1:1 CNC, 1:1:1 aa-CNC, 1:1:1 T-CNC) was studied in triplicate independently prepared samples. Hydrogel monoliths were prepared as described earlier, and 0.25 mL of a 100 mM NaCl/10 mM acetate buffer solution at pH 5 was added as preconditioning for 3 h in ambient conditions. This was followed by the addition of 0.25 mL 7200 ppm Cu²⁺ solution for 32 h in ambient conditions, after which the supernatants were removed and centrifuged for 10 min at 12,000g. 5 μ L aliquots of the supernatants were diluted 100-fold with water and then two-fold with the addition of PEI to 0.05 wt%, and analyzed for the [Cu²⁺] using the PEI-Cu binding assay as described in *Chapter 2 section 2.2.10 copper quantification through the PEI-Cu binding assay.* Briefly, PEI, a colorless polymer which turns bright blue upon complexation with Cu^{2+,30} was used to prepare calibration standards of PEI-Cu ranging from 0 to 50 ppm Cu²⁺ with a 0.05 wt% PEI, 0.25 mM NaCl and 0.025 mM acetate buffer matrix. Calibration standards and supernatant samples were pipetted into a Greiner UV-Star 96 well plate (200 µL), and absorbance at $\lambda_{max} = 273$ nm was recorded. A calibration curve was plotted using corrected absorbance, with 0.05 wt% PEI used as the blank (**Appendix A, Figure A11B**). A linear regression was used to determine the Cu²⁺ concentrations in the supernatant samples, accounting for the 200fold dilution during analysis. The metal sorption capacity at equilibrium (*q_e*) was calculated using **Eq 3.1**:

$$q_e = \frac{(C_i V_i - C_e V_T)}{m} \tag{3.1}$$

where $q_e (\text{mg} \cdot \text{g}^{-1})$ is the sorption capacity at equilibrium; C_i and C_e are the initial and equilibrium copper concentration (ppm); V_i and V_T is the initial and total volume of the solution; and *m* is the dry mass (g) of the sorbent. The average $q_e (\text{mg} \cdot \text{g}^{-1})$ was plotted for the four hydrogel compositions (1:3 H:C, 1:1:1 CNC, 1:1:1 aa-CNC, 1:1:1 T-CNC) where the data represents the mean and standard deviation from three independently prepared samples. Statistical analysis was performed on the data with the two-tail unpaired t-test used when comparing two independent compositions and the one-way ANOVA used for more than two independent compositions.

3.3 Results & Discussion

3.3.1 Characterization of Modified-CNCs

All starting reactive materials were synthesized in high yields with high purity and characterized in triplicate. The characterization of aa-HEC, aa-CMC and 4h-EDTA can be found in Chapter 2 section 2.3.1 quantification of aldehyde content on aa-HEC & aa-CMC. The aromatic aldehyde degree of functionalization (DoF) on the aromatic aldehyde modified-CNCs (aa-CNCs) was characterized using the same method as was used for the aa-HEC and aa-CMC polymer chains. The absorption spectrum for vanillin was compared to the absorption spectra of pristine CNCs and aa-CNCs, where a λ_{max} of 310 nm was found for both vanillin and the aa-CNCs while pristing CNCs showed no absorbance peaks (Appendix B, Figure B1). Thus, vanillin standards ranging from 0-60 μ M were used to make an absorbance calibration curve (Appendix A, Figure A2) to determine the aldehyde content on the aa-CNC. The concentration of vanillin was found to be 11 µM in 0.05 wt% aa-CNC samples. Assuming each cyanuric chloride was modified with a single vanillin molecule, the aa-CNC had a DoF of 0.0221 ± 0.0001 mmol aromatic aldehydes per gram of aa-CNC (~130 molecules of aromatic aldehydes per CNC particle). The carboxylated CNCs prepared via TEMPO-mediated oxidation (T-CNCs) were quantified for their DoF by conductometric titrations, which showed 2.27 ± 0.08 mmol carboxylic acid groups per gram of T-CNCs (~13,400 carboxylates per CNC particle; ~0.64 C/m²).

Furthermore, all three CNC types (pristine, aa-CNC and T-CNC) were characterized for their "apparent" diameter and surface charge using dynamic light scattering (DLS) and zeta potential (ZP), respectively. DLS gives the "apparent" diameter of the CNCs, which are rods while DLS assumes spherical particles. Therefore, these results mainly served as a comparison between samples and assessment of sample aggregation/size homogeneity. As expected, all samples

showed monodispersed sizes, and the aa-CNCs appeared larger than the pristine and T-CNCs, due to the aromatic aldehyde functionality (vanillin) grafted onto the primary hydroxyl group of anhydro glucose units via a triazinyl linker. The ZP serves as an indicator of colloidal stability, with larger magnitudes of ZP indicating more electric repulsion between adjacent particles in the suspension, which confers colloidal stability by resisting aggregation. As expected, the aa-CNCs showed the lowest ZP due to the aromatic aldehydes replacing surface hydroxyl groups, the pristine CNCs showed slightly higher ZP due to the presence of hydroxyl and sulfate half-ester groups, and the T-CNCs showed the greatest ZP due to the presence of negatively charged carboxylate groups alongside the sulfate half-ester and hydroxyl groups. The results obtained from these characterization techniques are summarized in **Table 3.1** for all CNC types.

Type of	Degree of Functionalization	Z-Average Diameter	Zeta Potential	
CNC	(mmol / g of CNC)	(nm)	(mV)	
Pristine	_	63.9 ± 0.3	-25.7 ± 0.3	
CNC				
aa-CNC	0.0221 ± 0.0001	76.0 ± 0.7	$\textbf{-24.8}\pm0.4$	
T-CNC	2.27 ± 0.08	63.9 ± 0.7	-29 ± 1	

Table 3.1. Summary of physiochemical properties of chemically modified-CNCs.

3.3.2 Qualitative Assessment of (Nanocomposite) Hydrogel Mechanical Strength via Swelling

Mechanically robust hydrogels able to withstand excessive swelling are a prerequisite for water treatment applications where the hydrogel must maintain structural integrity for facile handling during deployment into and recovery from water. Our previous findings from Chapter 2 discussed the importance of maximally incorporating aa-CMC into the hydrogel composition for its carboxylate groups, which result in high metal-binding capacity. We also highlighted the need to maintain a fraction of aa-HEC for its three-fold greater aromatic aldehyde content and absence of electrostatic repulsion on the polymer backbone, which ensures sufficient covalent crosslink formation. In this work, we opted to prioritize enhancing mechanical strength over metal binding capacity. We explored how the incorporation of pristine CNC, aa-CNCs and T-CNCs into the previously studied aa-HEC/aa-CMC hydrogel composition at a 1:1:1 aa-HEC/aa-CMC/(modified)-CNC ratio influenced the hydrogel's mechanical strength upon incubation in various ionic strength conditions and in excess Cu²⁺.

To determine the hydrogel composition that produces the most robust hydrogels at 2 wt% with a 1:2 aldehyde:hydrazide (a:h) crosslinking ratio, the 1:3 aa-HEC/aa-CMC (1:3 H:C) hydrogel composition was compared to three nanocomposite hydrogels (1:1:1 CNC, 1:1:1 aa-CNC and 1:1:1 T-CNC). These hydrogel compositions were studied for their swelling behaviors in three conditions: 25 mM NaCl/10 mM acetate buffer pH 5 (25/10 SB), 100 mM NaCl/10 mM acetate buffer pH 5 (100/10 SB), and 2500 ppm Cu²⁺ (post Cu). **Figure 3.1A** shows pucks for the four hydrogel compositions photographed before and after incubation in 100/10 SB and post Cu. From this qualitative data, the average normalized volume was determined assuming isotropic expansion (**Figure 3.1B**). The nanoparticle-free 1:3 H:C hydrogel puck shows the biggest expansion after incubation in the 100/10 SB and most shrinkage post Cu incubation. These results highlight the impact of carboxylate groups present in the hydrogel being the main source influencing hydrogel swelling. In all four hydrogel compositions, the aa-CMC biopolymer is the main source of carboxylate functionality, with the 1:1:1 T-CNC nanocomposite hydrogel having some contribution from the T-CNCs as well. While aa-CMC is present in all compositions, there is lower aa-CMC content in the nanocomposite hydrogels (33% aa-CMC) relative to the 1:3 H:C hydrogel (75% aa-CMC). Thus, the extreme swelling and shrinking behavior observed for 1:3 H:C in the 100/10 SB and post Cu incubation conditions, respectively, is expected. In 100/10 SB, the electrostatic repulsion from the negatively charged carboxylate groups causes the hydrogel to uptake water, and in post Cu, carboxylate groups coordinate to Cu^{2+} ions and cause water expulsion from the hydrogel.



Figure 3.1. Swelling of (nanocomposite) hydrogel pucks made at 2 wt% and 1:2 aldehyde:hydrazide crosslinking ratio. (A) Photographs of pucks made from 1:3 aa-HEC/aa-CMC (1:3 H:C), 1:1:1 aa-HEC/aa-CMC/CNC (1:1:1 CNC), 1:1:1 aa-HEC/aa-CMC/aromatic aldehyde-CNC (1:1:1 aa-CNC) and 1:1:1 aa-HEC/aa-CMC/carboxylated CNC (1:1:1 T-CNC).

Pucks were photographed before incubation, after 24 h incubation in 100 mM NaCl/10 mM acetate buffer pH 5 (100/10 SB), and after 1 week of incubation in 2500 ppm Cu²⁺ (post Cu). **(B)** Normalized hydrogel volume for all (nanocomposite) hydrogel compositions in various incubation conditions. <u>Note</u>: Line at 1 cm³/cm³ marks the pre-incubation puck volume. Data represents the mean and standard deviation for n = 4 independently prepared pucks. Statistical significance is presented as * p \leq 0.05, ** p \leq 0.01.

When comparing the average normalized volume, a one-way ANOVA test showed no significant differences between the control CNC, aa-CNC and T-CNC compositions for the 100/10 SB and post Cu incubation conditions. However, there was a significant difference for the 25/10 SB condition (Figure 3.1B), with 1:1:1 T-CNC showing a significantly larger normalized volume when compared to 1:1:1 CNC (p < 0.05) and 1:1:1 aa-CNC (p < 0.01). This indicates that the presence of carboxylate groups on the T-CNCs have a significant impact on hydrogel swelling in the 25/10 SB condition, but that the high ionic strength of the 100/10 SB successfully screens the surface charge and limits swelling. Thus, for the sorption capacity study discussed below, prior to adding excess copper, the 100/10 SB condition was used as the pre-conditioning solution to ensure charge screening of the carboxylate groups, and thereby maximal hydrogel stability. Additionally, for the 25/10 SB incubation, the 1:1:1 aa-CNC composition showed significantly smaller normalized volume than the 1:1:1 CNC control nanocomposite hydrogel (p < 0.01), suggesting that the aa-CNCs could form additional cross-links with the 4h-EDTA due to the aromatic aldehydes present on their surface. This hypothesis is also supported by the observation that the gelation time for 1:1:1 aa-CNC was faster than that of the 1:1:1 pristine CNC control (20 and 35 minutes, respectively).

Overall, the trend observed was that increasing the ionic strength from 25/10 SB to 100/10 SB decreased swelling of all pucks due to screening the charge from the carboxylate groups in CMC. On the other hand, incubation with excess Cu²⁺ resulted in the shrinking of the pucks from the carboxylate groups complexing Cu²⁺ ions. Generally, while the carboxylate content on aa-CMC influenced hydrogel swelling and shrinking, all nanocomposite hydrogels swelled and shrank significantly less than the 1:3 H:C hydrogel. This is because as there is less aa-CMC content in the nanocomposite hydrogels and the native or modified CNCs act as structural reinforcement, decreasing the influence of carboxylate groups and modulating water influx and efflux.

3.3.3 Quantitative Assessment of (Nanocomposite) Hydrogel Mechanical Strength

While the results from the swelling study offered insight into the improvement of the mechanical strength of the hydrogels upon addition of native or modified CNCs as green nano-additives, it was important to quantify the improvement in mechanical strength to better understand the mechanisms responsible for it. The storage (G') and loss (G'') modulus data is summarized in **Figure 3.2** for the 2 wt%, 1:2 a:h crosslinking ratio (nanocomposite) hydrogel pucks composed of 1:3 H:C, 1:1:1 CNC, 1:1:1 aa-CNC, and 1:1:1 T-CNC — where all compositions were tested before incubation (no SB), and after incubation in 25/10 SB, 100/10 SB and post Cu conditions.



Figure 3.2. Mechanical characterization of (nanocomposite) hydrogel pucks post incubation under various conditions. (A) storage modulus (G') and (B) loss modulus (G'') of pucks composed of 1:3 aa-HEC/aa-CMC (1:3 H:C), 1:1:1 aa-HEC/aa-CMC/CNC (1:1:1 CNC), 1:1:1 aa-HEC/aa-CMC/aromatic aldehyde-CNC (1:1:1 aa-CNC), and 1:1:1 aa-HEC/aa-CMC/carboxylated CNC (1:1:1 T-CNC). Pucks of all compositions were tested after incubation in four conditions: (i) 24 h in a humidified environment (no SB), (ii) 24 h in 25 mM NaCl/10 mM pH 5 acetate buffer (25/10 SB), (iii) 24 h in 100 mM NaCl/10 mM pH 5 acetate buffer (100/10 SB), and (iv) 1 week

in 2500 ppm Cu²⁺ (post Cu). <u>Note</u>: Data represents the mean and standard deviation for n = 4 independently prepared pucks. Statistical significance is presented as * $p \le 0.05$.

The storage modulus (G') showed the expected trend of nanocomposite hydrogels having greater mechanical strength relative to the CNC-free 1:3 H:C hydrogel (Figure 3.2A). The general trend for all compositions of hydrogels, 1:3 H:C being an exception, showed that increasing ionic strength of the incubation salt/buffer solution from 25/10 SB to 100/10 SB increased the mechanical strength of the hydrogels. This can be explained by the NaCl decreasing the electrostatic repulsion between the carboxylate groups. Interestingly, in all nanocomposite hydrogels there was a significant increase in G' upon incubating in SB versus the no SB incubation condition. We hypothesize that this is a result of all the hydrogel compositions swelling upon incubation, as discussed earlier, which could enable the dynamic hydrazone crosslinks to be formed optimally with rearrangement of active cross-link sites during hydrogel expansion. The only hydrogel that showed a different behavior is the 1:3 H:C composition, where the no SB incubation condition is significantly more robust compared to the 25/10 SB and 100/10 SB conditions. This is justified when looking at the swelling data, where the 1:3 H:C presented extreme swelling compared to the other compositions due to having the greatest carboxylate content. Based on these results, we propose that NaCl cannot penetrate fast enough into the 1:3 H:C hydrogel to enhance the mechanical strength by screening the carboxylates' negative charges and that the hydrazone crosslink density cannot withhold the initial influx of water. In conclusion, only the nanocomposite hydrogels hold potential as sorbents for heavy metal remediation of water since mechanical robustness is a key requirement for facile and reliable sorbent handling during sorbent deployment and sorbent recovery from water.

When comparing the G' values for the no SB, 25/10 SB and 100/10 SB incubation conditions between the nanocomposite hydrogels, one-way ANOVA showed significant differences in all individually assessed SB incubation conditions, with the G' for the T-CNC composition being significantly lower than that for the CNC and aa-CNC compositions. The lower mechanical strength observed for the T-CNC composition arises from the presence of the additional carboxylate content that causes increased electrostatic repulsion, greater water content and potentially reduced hydrazone crosslinking density. Although the mean G' value of the aa-CNC nanocomposites is greater than those containing CNC for all incubation conditions, the two-tail unpaired t-test between the paired treatments showed no significant difference. It is worth noting that while the qualitative swelling and gelation flip tests suggested additional covalent crosslinking in nanocomposites containing aa-CNC, the quantitative rheological measurements showed no significant increase in mechanical strength. Thus, the lower degree of puck swelling and faster gelation time for the aa-CNC composition, compared to the CNC control, were likely a result of hydrophobic interactions between the aromatic aldehydes and not from increased covalent crosslinking density. We concluded that the aa-CNC composition did not offer significant enhancement in the mechanical robustness of the nanocomposite hydrogel beyond the structural reinforcement provided by the pristine CNC control, so this composition was not explored further.

Lastly, we compared the G' values for the post Cu incubation condition. Although the T-CNC nanocomposite hydrogel was weaker relative to CNC and aa-CNC hydrogels in the SB conditions, it showed significantly larger G' values relative the CNC control hydrogels after incubating in excess Cu^{2+} (p < 0.05). Moreover, crosslinking via polyelectrolyte complexation from the interaction of Cu^{2+} with the anionic aa-CMC matrix,³¹ resulted in a significant increase in the mechanical strengths of all hydrogel compositions upon incubation with excess $Cu^{2+} - 3.4$, 5.1,

5.4 and 11.1-fold increase in G' for 1:3 H:C, 1:1:1 CNC, 1:1:1 aa-CNC and 1:1:1 T-CNC, respectively. This is a beneficial outcome for the application of heavy metal remediation since the hydrogel becomes more robust as water is expelled with metal binding. Additionally, when looking at the G" in **Figure 3.2B**, the only composition where the G" trend is opposite to that for G' is for the T-CNC nanocomposite hydrogel. Specifically, looking at the 25/10 and 100/10 SB conditions, the increasing G' and decreasing G" indicate the 1:1:1 T-CNC nanocomposite hydrogel becomes more elastic and less viscous due to water expulsion with increasing ionic strength. This follows the expectation that hydrogels with lower water content are more elastic and recover better after applying shear stress. Given that 1:1:1 T-CNC nanocomposite hydrogels are robust and easy to handle in no SB conditions and that they become stronger with exposure to metals (G' of 3100 Pa post Cu incubation), we determined them to be the most desirable for further exploration in heavy metal sorption applications.

3.3.4 Sorption of Copper onto (Nanocomposite) Hydrogel Monoliths

From our previous sorption studies on the 2 wt%, 1:2 a:h crosslinking ratio 1:3 H:C hydrogel monolith system, we found that an initial Cu²⁺ concentration above 200 mg/g was required to provide enough osmotic driving force for the ions to rapidly enter the hydrogel and overcome the mass transfer resistance between the liquid and gel phase. Thus, to assess the sorption capacity of the 1:3 H:C hydrogel against the nanocomposite hydrogels (1:1:1 CNC, 1:1:1 aa-CNC, 1:1:1 T-CNC), we used an initial Cu²⁺ concentration $C_i = 600$ mg/g. Prior to incubation with Cu²⁺, all the hydrogel monoliths were pre-conditioned in salt/buffer solution (100 mM NaCl/10 mM acetate buffer at pH 5) for 3 h. This was done to maintain the pH in the 5-6 range — high enough to deprotonate carboxylate groups on aa-CMC and T-CNC,³² while avoiding copper precipitation which is known to occur at pH > 6.³³

During the sorption study, we qualitatively tracked the Cu^{2+} binding progression over an excess time frame of 32 h to ensure equilibrium was reached between the sorbent (hydrogel) and sorbate (Cu^{2+}) (Figure 3.3A). From the profile photographs, we noticed the sorbate front moved at different rates for each composition. The sorbate front in the 1:1:1 CNC and 1:1:1 aa-CNC hydrogels moved fastest and at approximately the same rate, reaching the bottom of the monolith by 8.5 h, the 1:1:1 T-CNC nanocomposite hydrogel monolith required ~9.5 h, and the 1:3 H:C hydrogel monolith required ~ 10.5 h. These sorption rates correlated to the degree of carboxylate content present in the hydrogel monoliths, with compositions having greater carboxylate content experiencing slower sorbate front movement due to increased crosslinking via polyelectrolyte complexation. Thus, the 1:3 H:C monolith, composed of 75% aa-CMC, and 1:1:1 T-CNC, composed of 33% aa-CMC (like the CNC and aa-CNC nanocomposite hydrogels) with additional carboxylate content from the T-CNCs, experienced greater hydrogel crosslinking from carboxylates complexing Cu²⁺. This in turn increased the mass transfer resistance from the binding sites closest to the water interface forming a crosslinked surface and decreased the rate of sorption since excess Cu²⁺ cannot easily diffuse past the shrunken entry pores.



Figure 3.3. Sorption of copper by 2 wt%, 1:2 aldehyde:hydrazide crosslinking ratio (nanocomposite) hydrogel monoliths. (A) Sorption profile photographs of monoliths composed of 1:3 aa-HEC/aa-CMC (1:3 H:C), 1:1:1 aa-HEC/aa-CMC/CNC (1:1:1 CNC), 1:1:1 aa-HEC/aa-CMC/aromatic aldehyde-CNC (1:1:1 aa-CNC), and 1:1:1 aa-HEC/aa-CMC/carboxylated CNC (1:1:1 T-CNC) over 32 h at ambient conditions. Time point 0 h captures the monoliths preconditioned for 3 h with 0.25 mL 100 mM NaCl/10 mM acetate buffer solution (100/10 SB) prior to the addition of 0.25 mL 7200 ppm Cu²⁺ solution for 32 h. (**B**) The monoliths photographed at 0 h, before the pre-conditioning step and at 32 h after the removal of the excess Cu²⁺ solution. (**C**) Equilibrium sorption capacities (q_e) of (nanocomposite) hydrogel monoliths for incubation

with excess Cu^{2+} (t = 32 h). Data is presented as mean and standard deviation of n = 3 independent samples for each composition, and statistical significance is presented as * p ≤ 0.05 , ** p ≤ 0.01 .

Based on the qualitative observations of the rate of movement of the sorbate front, and the color of the monoliths upon removal of the excess Cu^{2+} incubation solution (Figure 3.3B), we predicted that the 1:3 H:C hydrogel monoliths would have the greatest binding capacity as it showed the slowest binding rate and the darkest color post-sorption, followed by the T-CNC, CNC and aa-CNC nanocomposite hydrogel monoliths. The equilibrium sorption capacity values, calculated by quantifying the unbound copper, are summarized in Figure 3.3C and match our prediction. The 1:3 H:C composition had the highest sorption capacity followed by the T-CNC, CNC and aa-CNC nanocomposite hydrogel compositions (110 ± 6 , 90 ± 10 , 70 ± 20 and 70 ± 10 mg/g, respectively). When statistical analysis was performed, one-way ANOVA showed a significant difference in the equilibrium sorption capacities. The two-tail unpaired t-test revealed that the binding capacity of the 1:3 H:C hydrogel was significantly greater than the CNC (p < 0.05) and aa-CNC (p < 0.01) nanocomposite hydrogels, as predicted to be the case since aa-CMC is the biopolymer responsible for binding heavy metals via the carboxylate groups. Therefore, decreasing the aa-CMC content from 75% to 33% in the nanocomposite hydrogels resulted in lower binding capacity. The T-CNC nanocomposite hydrogel showed a significantly higher binding capacity relative to the aa-CNC one but not the CNC nanocomposite hydrogel. This can be attributed to the CNC nanocomposite hydrogel having more available/accessible Cu²⁺ coordination sites than the aa-CNC nanocomposite hydrogel, which has fewer hydroxyl groups post-aromatic aldehyde modification compared to pristine CNC. Lastly, there was no significant difference in the equilibrium sorption capacities of the 1:3 H:C hydrogel ($110 \pm 6 \text{ mg/g}$) and the 1:1:1 T-CNC nanocomposite hydrogel $(90 \pm 10 \text{ mg/g})$. Therefore, as the 1:1:1 T-CNC nanocomposite hydrogel presents comparable

binding capacity to our past work while offering enhanced mechanical strength — a pre-requisite to templating microbeads — moving forward T-CNCs can be explored further as the green nano-additive of choice to simultaneously enhance binding capacity and hydrogel mechanical strength.

3.4 Conclusion

As heavy metal pollution in our water systems rises from increasing anthropological activities, cellulose-based nanocomposite hydrogel sorbents offer an economical, efficient and sustainable solution for heavy metal remediation. The goal of this work was to enhance the mechanical strength of our previous cellulose-based hydrogels, composed of two derivatives of cellulose which were functionalized with aromatic aldehydes (aa-HEC and aa-CMC), and crosslinked with an ethylenediaminetetraacetic acid (EDTA)-based small molecule modified with four hydrazide groups (4h-EDTA) at a 1:2 aldehyde:hydrazide crosslink ratio. This was done by exploring the addition of cellulose nanocrystals (CNCs) as green nano-additives in mixtures composed of 1:1:1 aa-HEC/aa-CMC/pristine CNC (1:1:1 CNC), 1:1:1 aa-HEC/aa-CMC/aromatic aldehyde-CNC (1:1:1 aa-CNC) and 1:1:1 aa-HEC/aa-CMC/carboxylated CNC (1:1:1 T-CNC). These nanocomposite hydrogels were compared to our previously investigated 1:3 aa-HEC/aa-CMC (1:3 H:C) composite hydrogel through swelling, rheology and sorption studies to determine the composition that produced the most mechanically robust nanocomposite hydrogel while achieving maximal binding capacity for Cu²⁺. All nanocomposite hydrogels showed lower swelling and greater mechanical strength compared to the 1:3 H:C hydrogel. This was attributed to CNCs being effective structural reinforcement agents and to the fact that there was lower aa-CMC content, which decreased the influence of carboxylate groups modulating water influx and efflux to and from the hydrogel. The swelling was further decreased in the presence of higher ionic strength incubation conditions of 100/10 SB and completely reversed in excess Cu²⁺ incubation conditions,

where all hydrogels experienced shrinking due to crosslinking via polyelectrolyte complexation from the interaction of Cu^{2+} with the anionic carboxylates in the matrix. Interestingly, rheological tests revealed all three nanocomposite hydrogels had a significant increase in storage modulus (G') upon incubating in salt/buffer conditions with respect to the no incubation condition, indicating that controlled/minor swelling could enable the dynamic covalent hydrazone crosslinks to be formed optimally with rearrangement of active cross-link sites during hydrogel expansion. The sorption capacity tests showed binding capacities of 110 ± 6 , 90 ± 10 , 70 ± 20 and 70 ± 10 mg/g for the 1:3 H:C, 1:1:1 T-CNC, 1:1:1 CNC and 1:1:1 aa-CNC hydrogels respectively. Of the three types of CNCs explored, the 1:1:1 T-CNC nanocomposite hydrogel - with a G' of 150 Pa enabling easy handling, and G' of 3100 Pa after incubating in excess Cu enhancing strength maximally — was determined to be the optimal composition for further exploration in heavy metal remediation applications. Overall, we showed that CNCs serve as effective green nano-additives for structural reinforcement of our previous cellulose-based hydrogels, with T-CNCs offering enhanced mechanical strength without losing significant binding capacity. Therefore, these nanocomposite cellulose hydrogels could serve as a green sorbent for the inexpensive and simple removal of heavy metals from water.

3.5 Acknowledgements

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Chapter 4: Conclusions & Future Work

4.1 Conclusions

The overarching goal of this work was to develop an economical, renewable, and biodegradable hydrogel able to efficiently bind heavy metals using the principles of green chemistry (7-use of renewable feedstock and 10-design for degradation). This was accomplished using cellulose — the most abundant biopolymer on Earth.¹ Specifically, cellulose derivatives, hydroxyethyl cellulose and carboxymethyl cellulose (HEC and CMC), were modified with aromatic aldehydes (aa-HEC and aa-CMC). These were covalently crosslinked with an ethylenediaminetetraacetic acid (EDTA)-based crosslinker modified with four hydrazide groups (4h-EDTA) to make hydrazone crosslinked hydrogels.

Chapter 2 explored these aa-HEC/aa-CMC/4h-EDTA cellulose hydrogels in-depth, where the main objectives covered were: (1) the synthesis of the hydrogel components; (2) determination of the aldehyde content on aa-HEC and aa-CMC; (3) optimization of hydrogel crosslinking ratio and composition using rheology — found to be 1:2 aldehyde:hydrazide (a:h) with a 2 wt% 1:3 aa-HEC/aa-CMC (1:3 H:C) composition having a storage modulus (G') of 200 Pa; (4) evaluation of the 1:3 H:C hydrogel's swelling and stability in different pH and ionic strength conditions — found to be stable in neutral and basic environments for one week, with higher ionic strength solutions slowing down bulk erosion via charge screening of carboxylate groups; and (5) evaluation of the 1:3 H:C hydrogel's physicochemical sorption properties via sorption isotherms, sorption kinetics, and a multi-metal sorption study with heavy metal ions (Cu²⁺, Zn²⁺, Co²⁺, Ni²⁺ and Mg²⁺) tested individually or as a multiple-component system — found Cu²⁺ to have the highest binding affinity and a maximum sorption capacity of 102 mg/g, comparable to other biosorbents. Overall, the

findings in Chapter 2 provided us with a better understanding of our cellulose-based hydrogel. We also observed an interesting interfacial phenomenon of rapid crosslinking of external hydrogel layers upon exposure to heavy metals. This led us to deduce key areas for further improvement, including the need to improve hydrogel mechanical strength and increase specific surface area to enhance sorption capacity and reduce sorption times. Thus, the main goal addressed in Chapter 3 was improving the mechanical integrity of our optimized 2 wt%, 1:3 H:C, 1:2 a:h hydrogel system while continuing to apply the principles of green chemistry.

In Chapter 3, we investigated the addition of (modified)-cellulose nanocrystals (CNCs) as green nano-additives — known to have high mechanical strength (elastic modulus of 143 GPa, comparable to Kevlar),² to improve the structural integrity of our previously optimized aa-HEC/aa-CMC hydrogel matrix. More specifically, we studied the incorporation of (modified)-CNCs at a 1:1:1 aa-HEC/aa-CMC/(modified)-CNC ratio using our 4h-EDTA crosslinker to form dynamic hydrazone bonds at the 1:2 a:h crosslinking ratio. Pristine CNCs served as a control and the modified-CNCs included aromatic aldehyde modified-CNCs (aa-CNCs) and carboxylated CNCs (T-CNCs). These three nanocomposite cellulose hydrogels (1:1:1 CNC, 1:1:1 aa-CNC, and 1:1:1 T-CNC) were tested alongside the 1:3 H:C composition for their swelling and subsequent rheology post incubating in salt/buffer conditions and in excess Cu²⁺. All nanocomposite hydrogels showed lower swelling and greater mechanical strength compared to the 1:3 H:C hydrogel post salt/buffer incubation. Additionally, after incubation in excess Cu²⁺, all hydrogel compositions experienced shrinking from polyelectrolyte complexation of Cu²⁺ with the anionic carboxylates in the hydrogel matrix. This significantly enhanced their mechanical strength, with the 1:1:1 T-CNC gaining the most strength — G' of 150 Pa pre-incubation to 3100 Pa post-excess Cu²⁺. Furthermore, sorption studies done using excess Cu²⁺ revealed the 1:1:1 T-CNC composition had a binding capacity of 90 mg/g for Cu²⁺, comparable to the 1:3 H:C hydrogel (110 mg/g for Cu²⁺). Overall, the findings in Chapter 3 were valuable in providing us with a blueprint towards using CNCs as green nanoadditives for templating renewable, biodegradable, and mechanically robust nanocomposite cellulose hydrogels. Accounting for mechanical strength and binding capacity, the 1:1:1 T-CNC nanocomposite hydrogel was deemed the optimal composition for future development of nanocomposite cellulose hydrogel microbeads. These beads could serve as safe, sustainable, costeffective, and easy to handle alternatives to powdered nanosorbents for water purification of heavy metals at an industrial scale while continuing to implement the principles of green chemistry.

4.2 Future Work

Our present work has made considerable contributions to developing and understanding cellulose based (nanocomposite) hydrogels, while also highlighting limitations of the bulk hydrogel design. Areas identified for improvement include overcoming diffusion hinderance of the metal ions to the coordination sites, and the need to improve binding affinity and efficiency. Therefore, future goals include (1) further studying (modified)-CNCs as green nano-additives to enhance hydrogel mechanical strength, (2) exploring microbead templating to have shorter diffusion pathways and for use in a packed-column system, and (3) investigating metal recovery via sorbent regeneration or degradation.

Regarding the first future goal, we could extend our work in Chapter 3 to investigate other ratios of the (modified)-CNCs. For example, we could consider 1:1:2 T-CNC to achieve potentially greater binding capacity while further enhancing mechanical strength. Another way we could incorporate CNCs into the aa-HEC/aa-CMC hydrogel matrix is as a crosslinker. This could be done using periodate oxidized CNCs where the bond between carbon two and carbon three of the anhydro glucose unit is cleaved to yield two aldehyde functional groups, thus referred to as

aldehyde modified-CNCs (a-CNCs).³ The a-CNCs could be reacted with excess 4h-EDTA to obtain a 4h-EDTA modified a-CNC (4h-EDTA_a-CNC) (**Figure 4.1**). This could serve as a mechanically robust nanoparticle crosslinker able to form hydrazone bonds with the aa-HEC/aa-CMC hydrogel matrix while potentially improving hydrogel mechanical strength and porosity.



Figure 4.1 Cartoon depiction of the 4h-EDTA_a-CNC crosslinker with the aldehyde groups on a-CNC (purple moiety on nanoparticle) reacted with excess 4h-EDTA molecules to form hydrazone bonds with a single hydrazide group on 4h-EDTA (blue corner on black star), where the other hydrazide groups remain available for crosslinking.

The second future goal of templating microbeads would be possible upon optimizing the hydrogel composition to have both competitive binding capacity and high mechanical strength. Making microbeads would increase the solution-gel interfacial area and thereby improve metal ion diffusion and chelation. This would result in faster metal scavenging kinetics and reduced regeneration times for potential reuse. Templating of microbeads could be achieved via either emulsion methods or a T-junction microfluidic device as previously done in the Moran-Mirabal research group by Levin et al.⁴ Furthermore, the development of nanocomposite hydrogel microbeads would also facilitate an easier shift from static batch experimentation to a dynamic column setup. The packed-column system would be transferrable to existing industrial water

treatment operations and equipment,⁵ offering the potential to develop these green sorbents into industrially employable materials.

The third future goal is to explore the life cycle assessment of our optimized hydrogel sorbent system and its end-of-life handling/processing protocol. While reusability is key for non-green sorbents to justify their environmental and monetary expenses, this is not as significant for biorenewable, biodegradable and inexpensive sorbents. With the economic value of metals contained in globally generated e-waste in 2022 estimated at 91 billion USD,⁶ there is great potential for metal recovery and recycling. And, for designing circular economy solutions, single use biosorbents could potentially be superior to multi-use synthetic sorbents. Thus, we would do reusability testing to gain understanding and insight into our hydrogel's ability to regenerate its binding sites but would be more interested in pursuing the single-use avenue which offers efficient recovery of the sorbed heavy metals. Unlike conventional recovery methods which use large volumes of washing agents and multiple pH adjustments, pyrolysis is a cost-effective and environmentally friendly approach to recover metals from used sorbents. Additionally, costs were compared for recovery of lead per ton of wastewater via biosorption-pyrolysis (\$0.06) and biosorption-desorption (\$0.19 using HCl or \$4.41 using EDTA).⁷ Therefore, taking into consideration the crosslink chemistry of our hydrogel where hydrazone bonds are known to be stable in pH ranges of 5-9,⁸ it would be possible to use either pyrolysis or acid degradation as costeffective and efficient methods to retrieve the sorbed metal. This would also limit hydrogel waste products to non-toxic biochar or small concentrated volumes of acid which could be neutralized. Overall, these next steps of further strengthening our hydrogels, templating microbeads, and assessing heavy metal recovery methods would guide these nanocomposite cellulose hydrogels

towards sustainable and affordable solutions for heavy metal remediation of water at an industrial scale with global applicability.

4.3 References

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Appendix A: Chapter 2 Supplementary Information

Supplementary Information for Chapter 2: Tetrahydrazide-EDTA-Crosslinked Cellulose Hydrogels for Water Treatment by Heavy Metal Chelation

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Scheme A1. One-pot synthesis of aa-HEC and aa-CMC.



Figure A1. ¹H NMR (600 MHz, D₂O) spectrum of (A) aa-HEC and (B) aa-CMC.



Figure A2. Vanillin calibration curve (0-60 μ M) made using corrected absorbance at $\lambda_{max} = 310$ nm with water as the blank.



Scheme A2. Synthesis of tetrahydrazide-EDTA (4h-EDTA).



Figure A3. (A) ¹H NMR (600 MHz, CDCl₃) and **(B)** ¹³C NMR (151 MHz, CDCl₃) spectrum of EDTA tetraethyl ester.



Figure A4. (A) ¹H NMR (600 MHz, D₂O) and (B) ¹³C NMR (151 MHz, D₂O) spectrum of tetrahydrazide-EDTA (4h-EDTA).



Figure A5. FTIR spectra of EDTA, EDTA tetraethyl ester and tetrahydrazide-EDTA (4h-EDTA).



Figure A6. ESI-MS $(M+H)^+$ of (A) EDTA tetraethyl ester (405.3, Na-adduct: 427.3) and (B) tetrahydrazide-EDTA (349.3, Na-adduct: 371.3).



Figure A7. Determination of the mechanical properties of aa-HEC/4h-EDTA 2 wt% hydrogels. (A) Representative shear test performed on a pre-gelled 4:1 aldehyde:hydrazide (a:h) puck. (B) Representative frequency sweep performed on a pre-gelled 1:4 a:h puck. Representative frequency sweep for the (C) storage (G') and (D) loss modulus (G'') of the in-situ gelled materials with varying a:h content.



Figure A8. Determination of the mechanical properties of aa-HEC/aa-CMC/4h-EDTA 2 wt% hydrogels. Representative frequency sweep for the (**A**) storage (G') and (**B**) loss modulus (G") of the pre-gelled 1:2 a:h pucks with varying aa-HEC/aa-CMC (H/C) content. Representative frequency sweep for the (**C**) storage and (**D**) loss modulus of the pre-gelled 1:2 a:h 25/75 H/C pucks after 24 h incubation in various solutions (excess Cu²⁺ solution of 208 mg Cu²⁺/g hydrogel; no solution control; 10 mM pH 7 phosphate buffer with 0 mM NaCl; 10 mM pH 7 phosphate buffer with 100 mM NaCl).



Figure A9. Hydrogel stability over six weeks. Plot of normalized hydrogel diameter (**A** and **B**) and mass (**C** and **D**) in various incubation conditions: 10 mM buffers with 0.02 wt% NaN₃ at pH 3, 7 and 11 with 0 mM NaCl, and pH 7 phosphate buffer with 0, 10, 50 and 100 mM NaCl for 2 wt%, 1:2 a:h, 25/75 aa-HEC/aa-CMC/4h-EDTA pucks; and pH 7 phosphate buffer with 0 mM NaCl for 2 wt%, 1:2 a:h, aa-HEC/4h-EDTA pucks referred to as "pH 7 control". Note: All error bars represent a standard deviation of n=3 independently prepared samples.



Figure A10. Calibration curves for heavy metal standards. Standards ranging from 0-100 ppm in 0.2 wt% HNO₃ and 1 mM NaCl/0.1 mM acetate buffer or salt/buffer (SB) matrix for (A-E) Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺ and Mg²⁺, respectively, obtained via ICP-OES.

Table A1: Validation of Cu²⁺ quantification by PEI-Cu assay using gold standard technique ICP-OES.

ICP-OES	UV-Vis
(mg/g)	(mg/g)
81	79
83	82
77	74
73	72



Figure A11. PEI-Cu binding assay. (A) Spectra of Cu^{2+} ranging from 0-50 ppm in a 0.05 wt% PEI, 0.25 mM NaCl and 0.025 mM acetate buffer (or salt/buffer; SB) matrix was used to create (B) a calibration curve for the PEI-Cu binding assay, obtained by UV-Vis absorbance (200 μ L/well).



Figure A12. Sodium content and volume retained by 2 wt%, 1:2 a:h, 25/75 H/C aa-HEC/aa-CMC/4h-EDTA hydrogels during a sorption isotherm experiment. Hydrogel volume change tracked by mass at each sorption isotherm concentration. Na⁺ mole ratio of final supernatant: initial added NaCl content measured via ICP OES using the supernatant samples at each sorption isotherm concentration.

Calculation of Degree of Functionalization: Determining the aromatic aldehyde content from

the vanillin concentration obtained via UV-Vis measurements at 310 nm.

- 1. Determine the concentration of vanillin in the aa-HEC using the calibration curve.
- Find the moles of vanillin: n = C•V. These are the moles of aldehyde for the specific wt% and volume of aa-HEC assessed (ex. 200 μL 0.05 wt%).
 ex. for 32 μM: n = (32x10⁻⁶ M)•(2x10⁻⁴ L) = 6x10⁻⁹ mol
- 3. To find mol aldehyde / g aa-HEC, divide by the mass of aa-HEC that was used to get the absorbance reading.

ex. for 200 μ L 0.05 wt% aa-HEC: 6x10⁻⁹ mol / 0.0001 g = 0.06 mmol CHO / g aa-HEC

4. To find the aldehyde degree of functionalization (DoF), isolate for x as shown in the figure below, where the triangle is the mol of aldehyde / g of aa-HEC.

 $0.00006 \text{ mol} / g = (x) / (x \cdot 245 \text{ g/mol} + 250 \text{ g/mol})$ x = 0.016 There are about **1.6 aldehydes per every 100 glucose units.**

5. To find the moles of aldehyde for 1 mol of aa-HEC, you need to find how many aldehydes are present per HEC chain.

ex. for a 90,000 Da HEC: 90,000 g/mol / 250 g/mol= 360 units long Thus, 1.6 aldehyde/100 units x 360 units = 5.8 aldehyde per aa-HEC chain This means there are **5.8 mol of aldehyde for 1 mol of aa-HEC**.

NOTE: for a 90,000 Da CMC, 445.77 units per chain is used (since the CMC is 70% carboxylated one unit is an average of $0.7 \cdot 219 \text{ g/mol} + 0.3 \cdot 162 \text{ g/mol} = 201.9 \text{ g/mol}$.

6. To find mmol of aldehyde/g of aa-HEC: (1g / 90,000 g/mol) • (5.8 mol CHO/mol aa-HEC)•10³ = 0.06 mmol CHO / g aa-HEC.



Appendix B: Chapter 3 Supplementary Information

Supplementary Information for Chapter 3: Nanocomposite Cellulose Hydrogels as Mechanically Robust Sorbents for Heavy Metal Remediation of Water



Figure B1. Representative UV-Vis spectra used to determine aldehyde content on aa-CNC. Spectra include vanillin (40 μ M), aa-CNC (0.05 wt%), and unmodified CNC (0.05 wt%), where a λ_{max} of 310 nm is used to calculate the degree of functionality on aa-CNCs.



Figure B2. Representative conductometric titration curve used to determine content of carboxylic acid groups on the surface of T-CNCs. The equivalence volume was determined by subtracting the volumes of the NaOH_(aq) at the two intersections and was used to calculate the moles of surface carboxyl groups. The first intersection is associated with the neutralization of the free $HCl_{(aq)}$. The second intersection represents the complete deprotonation of the carboxyl groups. Thus, beyond the second equivalence point, further titration with standardized NaOH results in an increase in conductivity due to the presence of excess NaOH_(aq). Overall, the conductivity starts off high, then drops to a plateau, and then rises due to: excess strong acid (excess H⁺), neutral T-CNCs (where all the T-CNC counter ions are now Na⁺) and excess base (free OH⁻), respectively. Note that as excess NaOH is added, free OH⁻ ions increase the conductivity, but the basic region is not as steeply changing as the acidic region since protons are more conductive than hydroxide groups.

Protocol to determine the moles of functional group content on the surface of T-CNCs (mmol COOH / g of T-CNCs)

- Find the equations of the three portions of the titration curve (down = acidic region, flat = neutral region, and up = basic region).
- 2. Equate the equations and find the points of intersection—the equivalence points.
- Take the difference of the equivalence points to obtain the volume of NaOH (aq) used to neutralize the T-CNCs (Ep2 - Ep1).
- Calculate mmol of COOH / g of T-CNC = [1000 x Concentration of Standardized NaOH x Volume of NaOH added (i.e. V2 - V1)] / [Mass of T-CNCs x (wt % / 100 %)]



Figure B3. Representative Dynamic Light Scattering Plots. (A) Pristine CNCs, **(B)** aa-CNCs, and **(C)** T-CNCs at 0.025 wt% in 10 mM NaCl (n=3).



Figure B4. Representative Zeta Potential Plots. (A) Pristine CNCs, **(B)** aa-CNCs, and **(C)** T-CNCs at 0.025 wt% in 10 mM NaCl (n=3).