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Acknowledgment

My journey in completing this thesis has been supported by many, but at the foundation of it all lies the unwavering spiritual guidance of Mahadev, the supreme source of strength, serenity, and inspiration. His divine blessings have guided me through the most challenging phases of this academic journey, giving me the clarity, endurance, and wisdom to complete this thesis with dedication and devotion.

I extend my heartfelt gratitude to my supervisor, Dr. Prince Chawla, for his insightful guidance, constant encouragement, and patience throughout the research process. Their mentorship has been a cornerstone of my academic growth, and I am sincerely thankful for their trust and support.

I would like to express my sincere appreciation to the School of Agriculture, Lovely Professional University, for providing me with a dynamic and resourceful academic environment. I am grateful to the faculty members of the Department of Food Science and Technology for their continuous support, expert knowledge, and motivation during my postgraduate journey. Special thanks to the non-teaching and laboratory staff who helped me during practical sessions and experimental setups. I would also like to thank the National Institute of Urban Affairs (NIUA) and my mentor Dr. Uday Bhonde for their association and any support or exposure that contributed to my research or professional learning. Working in collaboration or alongside such reputed institutions has enriched my perspective and broadened my understanding of real-world challenges and interdisciplinary approaches.

My warmest thanks go to my seniors for their guidance, encouragement, and willingness to share their experiences, which helped me avoid many hurdles. Their support was vital during my early research days and continues to be deeply appreciated.

To my dearest friends and classmates, thank you for being my support system, my partners in long lab hours, and my family away from home. Your laughter, motivation, and companionship made the toughest days lighter and this academic journey memorable. I will always cherish the moments of joy, teamwork, and friendship we shared.

I am especially grateful to my family, the backbone of my life. My parents have been my greatest source of inspiration and strength. Their unwavering faith, unconditional love, and endless sacrifices have been the foundation of all my accomplishments. You both have instilled in me the values of discipline, humility, and resilience. To my siblings, thank you for being my cheerleaders, advisors, and best friends. Your emotional support and constant motivation uplifted me during moments of doubt and fatigue. Your belief in my abilities pushed me forward and helped me stay strong. Lastly, I would like to acknowledge everyone who, in one way or another, contributed to the successful completion of this thesis, whether through a word of encouragement, a shared smile, or a quiet prayer. Every gesture of support, no matter how small, has meant the world to me. This journey has been a blend of challenges, growth, learning, and transformation and I am truly humbled and grateful for every part of it.

Abstract

Biopolymeric adsorbent materials, derived from renewable sources, have garnered significant attention for their eco-friendliness, biodegradability, and cost-effectiveness. Among these, starch stands out as a promising base material for fabricating advanced nanohydrogel adsorbents due to its modifiable molecular structure, biocompatibility, and high adsorption efficiency. In this context, the present study explores the sustainable rejuvenation of polluted ponds in Himachal Pradesh using maleic acid-modified red cowpea starch-based nanohydrogels. The native red cowpea starch demonstrated a high yield (35.12 ± 0.07%) and amylose content (33.00 ± 0.14%), which were altered upon modification (yield retained, amylose reduced to 27.65 ± 0.27%) to enhance its functional and physicochemical properties.Starch-based nanohydrogels developed from this modified biopolymer presented tunable porosity, high water absorbency, and broad-spectrum pollutant binding, making them effective for removing dyes, heavy metals, and organic contaminants from pond water. The study demonstrates that such green-engineered starch nanohydrogels offer a viable solution for the ecological restoration of stagnant and polluted water bodies. This sustainable and scalable approach aligns with circular bioeconomy goals and represents a transformative strategy for water body rejuvenation in Himalayan rural regions.

Chapter 1

Introduction

CHAPTER 1

1. Introduction

Water pollution has become one of the most critical environmental issues in recent decades, primarily due to the uncontrolled discharge of organic and inorganic contaminants into aquatic ecosystems (Ramirez-Gutierrez, Contreras-Jiménez, and Londoño-Restrepo 2024). Rapid industrialization, agricultural runoff, and domestic effluents contribute significantly to the release of harmful pollutants such as dyes, pesticides, pharmaceuticals, and heavy metals into water bodies. These contaminants are responsible for various health hazards, including waterborne diseases like cholera and hepatitis, as well as long-term ecological damage such as habitat destruction and developmental disruptions in aquatic life. The removal of such pollutants from water is, therefore, essential to protect human health and maintain environmental sustainability (Dehkordi et al. 2024). Among the numerous treatment strategies employed to mitigate water pollution, such as ion exchange, reverse osmosis, and biological treatments, adsorption has gained considerable attention due to its high removal efficiency, operational simplicity, and cost-effectiveness. Moreover, adsorption minimizes the generation of toxic by-products, making it a cleaner alternative compared to other conventional treatment methods. However, the efficiency of this process largely depends on the nature and properties of the adsorbent material used (Asadi-Ghalhari, Usefi, and Mahmoodi 2024). Ideal adsorbents should possess a large surface area, high mechanical and chemical stability, excellent binding affinity, and sustainability. In this context, biopolymer-based materials derived from renewable sources are gaining traction as eco-friendly and efficient adsorbents for water treatment applications.

Natural biopolymers like polysaccharides, proteins, and pectin exhibit desirable properties such as renewability, biodegradability, and functional modifiability, making them potential candidates for adsorbent materials. Among them, starch stands out due to its widespread availability, biocompatibility, and rich hydroxyl group content, which enables effective interactions with various pollutants. Despite these advantages, native starch presents several limitations, including high crystallinity, hydrophilic nature, poor thermal stability, and solubility issues. These drawbacks reduce its adsorption efficiency and structural integrity in aqueous environments.To address these challenges, starch modification has emerged as a promising research direction. Modification techniques aim to enhance starch's physicochemical, thermal, and functional properties, making it more suitable for environmental and industrial applications (Samaranayaka and Khazaei 2024). These techniques can be broadly classified into physical, chemical, and enzymatic modifications. Among them, chemical modification has been particularly successful due to its ability to introduce new functional groups into the starch molecule, thereby enhancing properties such as solubility, thermal stability, retrogradation resistance, and overall structural robustness(M. Sharma, Bains, Dhull, et al. 2024).

Esterification, oxidation, and cross-linking are common chemical modifications, with esterification using organic acids gaining significant interest for its green and sustainable nature. Maleic acid a dicarboxylic acid with two carboxyl groups—has proven to be an effective cross-linker for starch. Maleic acid reacts with the hydroxyl groups of glucose units in the starch backbone to form covalent ester bonds, resulting in enhanced hydrophilicity, ionization capacity, thermal stability, and water solubility (G. D. Singh et al. 2009). Unlike acetic or citric acid, maleic acid provides a higher degree of substitution and multiple ester linkages, contributing to improved functional performance and resistant starch formation. These attributes make maleic acid-modified starch highly desirable for industrial applications, including food packaging, pharmaceuticals, and environmental remediation(Soetan, Falola, and Nwokocha 2017).

In recent years, nanotechnology has further expanded the potential of biopolymers by enabling the development of polymer-based nanohydrogels. These nanostructured hydrogels offer a three-dimensional network capable of trapping and releasing contaminants through hydrogen bonding, ionic interactions, and covalent binding. Due to their nanoscale size and high porosity, nanohydrogels exhibit a larger surface area and higher adsorption capacity compared to conventional bulk adsorbents like beads or films. When starch is used as the base material for these nanohydrogels, it combines biodegradability and low-cost advantages with high functional tunability(T. B. Costa et al. 2024). These starch-based nanohydrogels can be tailored using additives, reinforcing agents, and fillers to improve their water absorption, mechanical strength, and pollutant-holding capabilities. Despite the abundance of starch sources such as corn, potato, and cassava, their extensive use raises concerns related to food competition, monoculture farming, and ecological imbalance(De Souza et al. 2025). This necessitates the exploration of underutilized, climate-resilient crops as alternative starch sources. One such crop is cowpea (*Vigna unguiculata*), commonly known as lobia or red-eyed bean. Belonging to the Fabaceae family, cowpea is extensively cultivated in Sub-Saharan Africa, Asia, and parts of Europe. It is well adapted to arid and semi-arid conditions, making it a sustainable crop option for regions affected by water scarcity and soil salinity(Ritte et al. 2025). Referred to as the "poor man's meat," cowpea is nutritionally rich, containing 50–65% carbohydrates and 15–30% proteins. Despite its nutritional and industrial potential, cowpea remains underutilized, especially as a starch source.

The starch content in cowpea ranges from 25% to 45%, and it exhibits a low glycaemic index, high resistance to digestion, and several health benefits, including antioxidant and anti-inflammatory effects. Extracting starch from cowpea not only offers a sustainable alternative to conventional starch sources but also contributes to the circular economy by utilizing an underexploited resource(B. Kumari and Sit 2024). Starch extraction methods include wet and dry milling, enzymatic treatments, and other non-conventional techniques involving suspension, centrifugation, and drying. These methods need optimization based on the source material and intended application to ensure high yield and purity. Modification of cowpea starch, particularly through esterification using maleic acid, significantly improves its functionality(Ghaffar et al. 2025). This modification introduces carboxyl groups that enhance the starch’s ability to bind with pollutants and biological molecules, making it suitable for applications like controlled release systems in pharmaceuticals or as bioactive food ingredients.This research, therefore, aims to explore the extraction, modification, and characterization of red cowpea starch using maleic acid, with a particular focus on its antioxidant, anti-inflammatory, and in-vitro digestibility properties. Additionally, the study evaluates the application of maleic acid-modified starch as an effective adsorbent for dye reduction and heavy metal detection in water purification systems. By demonstrating the multifunctional capabilities of red cowpea starch, this study contributes to the broader goal of promoting sustainable materials for environmental and health-related applications.

In conclusion, the aim of this study is to provide an approach for enhancing the value of starch through eco-friendly extraction and modification using organic acids, thereby enabling its potential use in the environmental and food sectors to contribute to fulfilling the global sustainable development goals. The modified cowpea starch has much-improved qualities, it has numerous applications where thermal stability, functional group availability, and particle behavior are required. Modified starch in the food industry helps to create products that are clean-label and functionally useful, due to its ability to form films and biodegradable nature, it is seen as an effective solution for making environmentally friendly products. Overall, maleic-acid MS is emerging as a sustainable and promising alternative with the potential for innovation in biodegradable packaging, food technologies, and other bio-based material solutions.

Chapter 2

Review Of Literature

CHAPTER 2

2. Review of Literature

2.1. Starch extraction through green technologies

It involves sustainable methods and procedures, minimizing environmental impact by eliminating waste, utilizing natural resources, decreasing pollution levels, and reducing the carbon footprint by 40% (Rashwan et al. 2024). Conventional methods involving, oxidation, wet milling, esterification, alkaline treatment, high pressure, etc. utilize chemicals, reduce efficiency, and result in less yield (Torres and De-la-Torre 2022). A study done on alkaline treatment, resulted in changing the functional and physicochemical properties of starch by breaking down the cellular matrix, disrupting the cellular structure, and releasing non-starchy components present in Figure 1A (Zekun Xu et al. 2024). Sodium hydroxide, potassium hydroxide, and sodium metabisulphite are the most used alkaline agents that change the granular and structural composition of the starch. According to Zhang et al. (R. Y. Zhang et al. 2024), shown in Table 1, tiger nut meal was treated with alkali treatments at different temperatures for six samples and starch yield was highest in the case of HNS-5 having 5g/L NaOH concentration at 36.2 % and lowest in the case of DWS at 23.3%, whereas the amylose ratio of these samples was between 9.3% – 13.7%. Whereas green extraction methods minimize the use of chemicals, are energy efficient, and environmentally friendly studied by Raspe et al. (Raspe, da Silva, and Cláudio da Costa 2022)include enzymatic-assisted extraction, ultrasound-assisted extraction, pulsed electric field, supercritical fluid technology, subcritical water extraction, and microwave-extracted technology (Marianne et al. 2024a). These non-conventional techniques increase the yield, lower the consumption of solvents, require little maintenance, provide economic benefits, conserve resources, and produce non-toxic produce.

Sources of Starch	**Extraction Methods**	**Yield**	**Composition**	**Reference**
Green Banana (Peeled and unpeeled)	Enzymatic treatment	Resistant starch Peeled- 88.7% Unpeeled- 85.62%	Amylose-48.53% for peeled Amylose- 43.41% for unpeeled	(Dong et al. 2024)
Yam	Ultrasound-assisted extraction	Starch by steeping in alkaline pH- 45.90±0.68% Acid pH- 50.86±1.23% And water- 41.23±4.92%	Amylose content Purple yam- 9.05% White yam- 6.93%	(Ling Chen et al. 2022; Liwei Chen et al. 2021)
Mango seed Kernel	Aqueous extraction	Starch 52.89% to 62.11%	Starch 52.89% to 62.11%	(Edo et al. 2024)
Tiger nut	Alkaline extraction	Starch- 36.2%	Amylose 9.71%-13.7%	(Castro et al. 2023)
Cassava	Enzymatic treatment	Starch- 30.17±2.18%	Amylose-21.70%	(El-habacha et al. 2023)
Corn	Enzymatic extraction	Starch- 65.18%	Amyolse-35.18%	(Zeyi Liu et al. 2024)
Oats	Subcritical water extraction	Starch-65%-70%	Amylose-30.12% (maximum at 115° C), Amylose- 31.68%	(S. G. Costa et al. 2024)
Taro	Enzymatic extraction	Starch- 17.22%	Amylose- 8.7%-14.9%	(Duquette and Dumont 2018)

Table 1. Extraction methods of starch

2.1.1. Ultrasound-assisted extraction

Ultrasound-assisted is an environmentally friendly, economical, and efficient technology that uses ultrasound leading to thermos-physical effect causing the disruption of cellular membrane by generating cavitation which forms bubbles in liquid and degrades hydrogen bonding, hydrophobic interactions, and van der Waals forces between molecules of solids mechanically for the extraction of various compounds as shown in Figure 1B (Kumar et al. 2024). This method uses less solvent than the traditional method, and the extraction rate and efficiency of extraction are increased. it also depends upon the time of solvent used, temperature, intensity of ultrasound, and solid-solvent ratio (Cui et al. 2022). Ultrasound-assisted extraction yield, as mentioned in Table 1, helps in extracting starch from anthocyanin residue obtained from purple yam from three methods, such as 1% ascorbic acid solution, 2.5% sodium hydroxide solution, and deionized water (Ochoa and Osorio-Tobón 2024). According to Shrivastava et al. (Shrivastava, Gupta, and Srivastav 2024), shown in Table 1, starch was 41.23- 50.86% overall, from alkaline pH extraction about 45.90%, the highest percentage, through ascorbic acid pH 50.86%, and from deionized water extraction 44.23% whereas the amylose content was high in number around 9.05% in purple yam and white yam consisting of 6.93% amylose. Hence, this method is efficient for increasing the yield, reducing the processing time, and minimizing the use of solvents.

2.1.2. Subcritical water extraction

This technique includes heating of water above the boiling point range of around 100-374°C and pressure under 22.12 MPa to remain in a liquid state which does not include the use of any solvent other than water, making it environmentally friendly. It is also effective for dissolving compounds with low polarity as water's hydrogen-bonded structure is disrupted at high pressure and temperature (Radovanović et al. 2022). As shown in Table 1, In a subcritical water reactor at temperatures i.e. 115°C the starch content was highest at 30.12%, and at 190°C starch content was 26.58%, which took place due to the easy release of amylose from the starch structure for 20min. under 40bar pressure for nitrogen gas, also it was observed that with increasing temperature the amylose content decreased (Surendhiran et al. 2023). According to Li et al. (Y. Li et al. 2022) studied the cold-water swelling ability and cold-water solubility of oats starch at various temperatures, showed the maximum rate at 15.72 g/g of 95ºC temperature with 68% ethanol concentration and declined at the highest temperature at 150ºC for 20min. as shown in Table 1, whereas the cold-water soluble ability was also highest at 95ºC but at 38% ethanol concentration i.e. 19.90% and lowest at 68% concentration at 1.52%. Thus, subcritical water extraction provides a sustainable approach by using environmentally friendly methods to create highly effective materials.

2.1.3. Pulsed electric field

A pulsed electric field uses solid, semi-solid, and fluid material where repeatedly high voltage (20-80kV/cm) of short pulses are applied between two electrodes. Pulsed electric field alternates with the magnetic field, which results in changes in the cell membrane's local structure and the membrane barrier is also damaged. It is a non-thermal processing technique that helps maintain the nutritional value and original colour of the food, and the short pulses control the undesirable effects due to temperature (Mudaliar et al. 2023). This technology is used in the food industry for various commodities as in fruit beverages, where a study done by Hou et al. (Hou et al. 2021), showed that carotenoid digestibility increased in milk by 15% when compared to milk not treated with pulse electric field. For extracting starch from macroalga coupled with biomass fractionation into protein and ash, pulsed electric filed is used by making a suspension of alga, treating it with electric filed then filtration of suspended thalli after agitation is done to obtain starch which is dried. In the case of acorn starch (Castro et al. 2024, 2023) shown in Table 1, starch yield came out at 34.5% at intensity of electric field at 2.86 and 50kV/cm and frequency of 600 and 1008Hz , and amylose content was 62.1% which showed an increase by 9%. This method improves the viscosity and swelling capacity of starch by preserving its native properties.

2.1.4. Microwave-assisted extraction

Microwave-assisted extraction involves the use of microwaves for extraction which are electromagnetic waves having wavelengths of 300MHz-300GHz. As shown in Figure 1C, it involves heating of solvent molecules through collision and friction, containing sample by using microwave energy, as microwave has strong penetration power and can easily penetrate cell walls and dissolute the polysaccharide resulting in less consumption of organic solvents and less extraction time which is economical (X. Chen et al. 2021). As shown in Table 1, it was observed that starch yield obtained was 49.52% at 161.09 °C for 56 min. and a wavelength of 2.45GHz , was more than the conventional method yield which helps in increasing the water absorption capacity and swelling power (González-Mendoza et al. 2022; Araújo et al. 2020). Thus, microwave-assisted extraction helps minimize the starch from gelatinizing and maintains the structure and functional properties of starch resulting in higher yield and less wastage of energy by reducing the time of the extraction process, it is an efficient technique than conventional extraction methods.

2.1.5. Enzyme-assisted extraction

Due to the complex structure consisting of hydrogen or hydrophobic bonds in the plant matrix, it is very difficult for a solvent to extract its compounds from the cell walls and other matrices. As shown in Figure 1D, enzymatic-assisted extraction is an eco-friendly technology that uses various enzymes such as amylase, xylase, pectinase, etc. for polysaccharide hydrolysis, as it takes less time, less solvent consumption, and extracts compounds of high quality (Dikmetas et al. 2024). According to a study done by Islam et al. (Islam et al. 2024), enzymatic treatment was given to green bananas for the isolation of resistant starch by using amylopollulanase and amyloglucosidase enzymes at pH 5.2 at 37 °C, relative starch of native green banana flour was maximum at 68.99%, whereas the amylose content of unpeeled banana resistant starch was minimum at 43.41%, also the starch yield of peeled banana was the maximum among other starch sources at 88.7%, whereas taro starch expected least yield of at 17.22% as well as least amylose content at 14.9% shown in Table 1. Enzymatic treatment was also given to corn kernels according to a study done by Liu et al. (Zeyi Liu et al. 2024) shown in Table 1, for extracting starch as the traditional method causes environmental pollution and low purity of starch, enzymatic extraction also increased the starch content by 30.45% by using protease 0.45% and L-cysteine at 0.45g at 6pH and temperature 40 °C from traditional extraction which is 30.73%, and amylose content at 35.18%. Another study done by Gupta et al. (Gupta, Guha, and Srivastav 2024; Thuppahige et al. 2023), in Table 1 showed that starch yield from taro and cassava came out at 17.22% and 30.17% after centrifugation at 3000g for 15min. after 3-4 times at 4.5-6.6 pH ,respectively whereas amylose content was 8.7%-14.9% in taro starch and 21.70% in cassava. 3Thus, enzymatic-assisted extraction offers a sustainable and efficient method for obtaining high-quality natural products and enhancing yield while minimizing the use of chemicals and environmental impact.

Fig. 1- Extraction methods of starch

2.2. Effect of green extraction technique on starch composition and chemistry

Starch molecules are bonded with glycosidic bonds composed of amylose with α-1,4-glycosidic bonds and amylopectin with α-1,6-glycosidic bonds, comes under reserve polysaccharide having crystalline and amorphous regions (Jia et al. 2023). Amylose has a linear structure along with less branching as compared to amylopectin which is highly branched and has nonrandom α-(1→6) linkages which helps the starch to attain its crystalline structure and unique texture(Adeleke Omodunbi Ashogbon 2021). For example, high amylose content (25-30%) gives a hard texture to cooked rice (below 20%) and amylose content provides a glossy and cohesive texture to cooked rice; whereas raw rice has 28.49% crystallinity which comes down to around 5.72% after steaming (Guo et al. 2023). Starch in its native form does not have good thermal and shear stability, has poor freeze-thaw stability, high retrogradation capability, and no effect due to enzymatic hydrolysis. To maximize the benefits of using starch for good adsorption capacity, modification needs to be done (Mhaske et al. 2022). In a study by (Baharuddin, Nik Sulaiman, and Aroua 2014), unmodified starch was used for the removal of chromium ions through ultrafiltration process at pH7 and retention by polyethylene glycol, which was dependent upon the granular structure of starch and not on starch type and also less concentration was required to overcome gelatinization which is not desired. Limitations of using unmodified starch were the hydroxyl group embedded within the granule of starch, which may not be accessible for binding of pollutants.

Native starches without the use of heat do not form a gel network for pollutant removal, which also results in poor adsorption kinetics (Liwei Chen et al. 2021). Upon comparing unmodified starch with modified starch, modified starch for adsorption serves more adsorption sites by introducing more specific functional groups for adsorption of various pollutants found in the water (Zheng et al. 2023). Starch has gained significant attention due to its diverse applications and has emerged as an exceptional base material such as tapioca starch, corn starch, potato starch, etc., providing support and enhancing the properties of different compounds (Metawea et al. 2023).

Various functional properties of starch such as gelatinization, retrogradation, solubility, biodegradability, viscosity, and crystallinity play an important role in determining its behavior during food processing properties (Zhuang et al. 2024).In the case of a study on legumes done by Obadi et al. (Obadi and Xu 2024) the elevated gelatinization temperature is thought to result from the high crystallinity and structural stability of the starch granules, which increases their resistance to gelatinization. Thus, the unique composition and structural characteristics of starch make it a versatile and valuable biomaterial with immense potential for innovative applications in various fields.

2.3. Preparation of Starch-based nanohydrogel using green approaches

Green methods focus on minimizing the use of harmful chemicals, reducing energy usage, and making use of renewable, biodegradable materials like starch, a natural polymer (Z. Yang, Xie, and Cai 2024). The preparation of starch-based nanohydrogels through green approaches ensures the development of highly efficient, biocompatible, and biodegradable materials with promising potential in applications such as targeted drug delivery, water purification, and sustainable agriculture. Green techniques include enzymatic processes, microwave-assisted synthesis, ultrasonication, and hydrothermal treatments (Ji et al. 2018). According to Qamruzzaman et al.(Qamruzzaman, Ahmed, and Mondal 2022) several modification techniques for starch, form the basis for nanohydrogel including physical modification which consists of two types such as thermal including hydrothermal process and annealing, and non-thermal treatment which involves ultrasonication, pulse electric field, high-pressure processing, and micronization (Dhull et al. 2024). These methods are cost-effective, reorganization of crystalline regions within the granules leading to increases in the swelling capacity, thermal stability, and pasting properties shown in Figure 2A. For instance, Barua et al.(Barua et al. 2024) explained the hydrothermal process used for elephant foot yam and cassava includes recrystallization at 4°C which helps the starch to exhibit higher resistance to heat during thermal processing and ultrasonication at 24kHz power decreases the particle size and improves the amylose content. According to Maqtari et al.(Al-Maqtari et al. 2023) and Bangar et al.(S. P. Bangar et al. 2022) physical methods increase the amorphous region and surface area for water interaction and increase amylose-lipid complexes by applying heat moisture treatment (Z. Wang et al. 2024).

Another technique is chemical modification involving oxidation, esterification, cross-linking, etherification, grafting, and dual modification which improves solubility, water binding capacity, and viscosity (L. Kaur, Kaur, and Singh 2024). It helps enhance the gelatinization tendency, increases the rate of conversion, optimizes functional and nutritional properties, and improves compatibility such as in some studies on cassava (L. Kaur, Kaur, and Singh 2024; Sumardiono et al. 2024) also shown in Figure 2B, explains hydrothermal esterification which includes the heating of starch that disrupts the structure to make the hydroxyl group more reactive with esterifying agents. Agents mostly used according to Maleki et al. (Maleki et al. 2024) are citric acid, oxidative cross-linking involves crosslinking between different starch molecules along with oxidizing agents. This method involves forming covalent bonds between polymer chains, conversion of hydroxyl groups into carbonyl or carboxyl, or rearranging or adding new functional groups to obtain better characteristics of starch, resistance to heat, acid, and shear (S. P. Bangar et al. 2022).

Lastly, enzymatic modification includes the use of enzymes such as amylase and glucoamylase to alter its structural, functional, and regulating physiochemical properties (A. K. Singh et al. 2021). Breaking down and modifying starch structure through hydrolysis reduces the viscosity, enhancing branch density in the case of amylase, and shortening external chains and internal chains in the case of maltogenic amylase as shown in Figure 2C (Wu et al. 2024).To modify the properties of starch molecules for various reasons such as to increase the adsorption capacity and encapsulation such as in the case of a banana studied by Garofalo et al. (Garofalo et al. 2024), heat moisture treatment was applied which enhanced the functional properties and increased the gelatinization temperature and relative starch thermostability. For corn starch modification by enzymatic treatment studied by(Sujka and Wiącek 2024; Palma-Rodríguez, Vargas-Torres, and Leyva-López 2024), amylase and pullulanase enzymes were used which break down branched starch molecules into linear form and help enhance the digestibility, increase solubility, and reduce the formation of by-products. Overall, modification is used to make nanohydrogel by physical, chemical, and enzymatic treatments which alter the starch properties through the introduction or rearrangement of function groups, reducing intermolecular hydrogen bonding.

2.3.1. Starch-based nanohydrogel vs. other adsorbents

Pollutants removal by using adsorption material was first discussed in the late 1910's in a research paper which suggested dye molecules separation with the help of sodium nitrate, lead nitrate, and barium nitrate (Chapman and Siebold 1912). For removing violet dye lead sulphate was used in another study along with crystal violet and Orange II were degraded by anatase, rutile and zinc oxide. However, it took almost nine days to degrade so, the research sifted towards material having more porous structures which resulted in kinetics of adsorption of methylene blue and sulphur blue dyes at 0 to 0.07 $mg \cdot g^{-1}$ min^{-1} and 16 $mg \cdot g^{-1} \cdot min^{-1}$ (Whetstone 1957). Around the early 21st century, the use of polysaccharide based adsorption materials such as rice starch resulted in greater adsorption than potato starch due to high affinity of pollutant adsorption and low cost for removal of acid dyes and heavy metals (Kyzas, Fu, and Matis 2013; Lloyd 1911). In terms of cost-efficiency, biodegradability, sustainability, and functional adaptability, starch-based nanohydrogels have an advantage over natural and synthetic biopolymers used as an adsorbent material (Gamage et al. 2022),however adsorption capacity of synthetic adsorbents resulted in better such, as in the case of malachite green dye adsorption by synthetic polymer $La(OH)_3$@SA/PAM at 3000mg/g (Junlapong et al. 2020). Biopolymer nanohydrogel chitosan-based nanohydrogel had 1275.9mg/g adsorption capacity in case of pesticides and organic pollutants when compared with other natural polymer-based nanohydrogel, such as Kaolinite at 22.89mg/g, NiO at 11.21mg/g, and synthetic polymer such as PANI-$NiFe_2O_4$ for heavy metal like Cr or Pb though cheap and abundant but lacks functional tunability (G. Sharma et al. 2017; Shang et al. 2024). A study by (Lee et al. 2018)done on synthetic biopolymers such as TiO_2/ SiO_2 resulted in high potential for pollutants at 5.93mg/g for methylene blue dye, but had limitations such as biodegradability, high cost, fewer scalability options, and toxicity, whereas starch adsorbent reflected excellent regeneration at high temperature and by multiple washings without losing its structural and functional properties(Akhtar, Ali, and Zaman 2024). Hence, starch-based adsorbents have economic feasibility, environmental compatibility, and make it highly suitable for pollutant removal.

Initial research on starch-based nanohydrogel synthesis has resulted in initiating the current studies for exploration of the potential application, properties, and unique characteristics of starch, which is a biodegradable, low-cost, and renewable material for nanohydrogel development (Saracoglu and Ozmen 2021). Studies based on nanohydrogels production initially by methods such as cross-linking, esterification, etc. such as in the case of drug delivery applications, and also helped to enhance their mechanical properties (Bakrudeen, Sudarvizhi, and Reddy 2016; Tan et al. 2009). In a study (Koshenaj and Ferrari 2024), done on structures, revealed the starch's amylose to amylopectin proportion, directly influencing the nanogel structures' formation and development. The properties of water absorption capacity and mechanical strength can be adjusted through modification of these components, and techniques such as High-Pressure Processing are used as a sustainable and green technology to modify starch structures for nanohydrogel development (An et al. 2023; Bakrudeen, Sudarvizhi, and Reddy 2016). Starch-based nanohydrogel applications for water treatment have shown advancement, which supports their practical use in large-scale operations. A study by (Ling Chen et al. 2022) on nanohydrogels by combining starch with carbon nanotubes into polyvinyl alcohol matrices demonstrated excellent water filtration abilities by photocatalytic effect and reached evaporation rates of 2.44 $kg/m^2/h$ and 95% efficiency. These materials efficiently extract heavy metals and dyes and organic pollutants together with antibacterial properties and durability, which enables them to work in wastewater treatment systems. The synthesis of hydrogels from gum tragacanth and starch resulted in a study by (S. Ahmad and Imran 2024) that reached optimal conditions for removing 97.6% of methylene blue and 93.7% of Congo red dye. The materials display exceptional pollutant removal abilities as they adsorb heavy metals, dyes, and pesticides, as well as pharmaceuticals, at rates exceeding 90% (Khoo et al. 2023). Further research through field-scale trials must confirm their actual operational performance. Studies continued to address the challenges of nanohydrogel instability as well as efficient water degradation because scientists need better functionality from starch-based nanohydrogels to apply them across different environmental needs (Dong et al. 2024; Nasrollahzadeh et al. 2021). These research demonstrate that starch-based nanohydrogels are becoming increasingly important because they provide sustainable solutions to manage global water scarcity and pollution.

2.3.2. Preparation of starch-based nanohydrogel: Methods and mechanisms

The preparation methods used to make starch and starch-based nanohydrogels present varying benefits together with corresponding constraints. The physical techniques of ultrasonication and microwave therapy together with high-pressure processing provide an eco-friendly approach that preserves starch’s natural structure for their application in food production and biomedical applications, these preparation procedures need high-energy machinery alongside specialized tools and demonstrate low effectiveness for material modifications (Singla and Sit 2021; Abdol Rahim Yassin et al. 2023). The use of acid/alkaline hydrolysis combined with boric or citric acid crosslinking and oxidation treatments changes starch structure which leads to better functional characteristics and high-water absorption (Malumba et al. 2022). These modified starches are extensively used in creating pharmaceutical systems for drug delivery, purifying water, and in biopolymer technology, however, chemical modification of starch causes toxic substances, require purification processes leading to environmental problems (S. Mishra, Chowdhary, and Bharagava 2019). The enzymatic process that utilizes amylase or lipase hydrolysis produces high purity modified starch while creating nanohydrogels with controlled properties through a biocompatible method, which operates with high selectivity and mild action, it possesses both high sustainability and precision, but operates at a slow pace and has expensive requirements (Zarski et al. 2024). Hence, the selection of procedure depends on the desired end use while considering cost and performance alongside efficient production and environmental consequences.

The selection of preparation methods for starch and starch-based nanohydrogels depends on specific applications for distinct advantages, such as ultrasonication and microwave treatment are ideal options for the food and pharmaceutical industries because their low toxicity meets safety standards and allows persistence of natural material properties for drug carriers and biodegradable food coatings (C. Sun et al. 2024; Zhao et al. 2024). Chemical crosslinking prepare starch-based nanohydrogels optimally for industrial and environmental applications that require durable water purification systems as well as packaging films and controlled drug delivery systems due to their enhanced stability properties, however this method is less suitable for food and biomedical applications because of its possible toxicity along with residual chemical, so it requires extensive purification (T. M. Ali et al. 2024). Enzymatic hydrolysis using amylase or lipase enzymes in medical hydrogels enables the delivery of tissue engineering structures and nutraceutical formulations that require high levels of biocompatibility and specificity, through this approach nanohydrogels provide high-grade products that are appropriate for use in wound healing applications and probiotic delivery systems, but limitations can include reduced hydrolysis efficiency and high cost of enzymes(Witasari et al. 2024) . The choice of procedure depends on multiple factors which consist of both safety aspects and cost efficiency together with environmental integrity and specific functional needs of the product.

Starch-based nanohydrogels develop their structural properties during preparation in a manner that determines their ability to extract dyes, pesticides, and microorganisms, and heavy metals and other pollutants. Nanohydrogels produced by ultrasonication and high-pressure homogenization methods become smaller with greater surface area that increases their ability to adsorb heavy metals and organic pollutants by improving both surface interaction and diffusion, however, the adsorption selectivity is restricted for unmodified nanohydrogels(Guida et al. 2024). The process of chemical crosslinking with acids forms stronger network structures for porosity control which improves selective adsorption of charged dyes and metal ions by electrostatic and chelation interactions, but residual chemicals from chemically crosslinked nanohydrogels could present toxicity issues while the structures benefit from better stability and reusability (Agha et al. 2025; Abdul Hameed, Al-Aizari, and Thamer 2024). Enzymatic modification that incorporates lipase or amylase-based hydrolysis enhances biocompatibility and surface hydrophilicity which makes nanohydrogels suitable for microbial adsorption and biofiltration usage, although the rate of reaction is slow, leading to weakened mechanical strength, which causes performance degradation during water treatment operations(Qiu et al. 2023; Sellami et al. 2021). Nanohydrogels that use graft polymerization and nanoprecipitation methods exhibit remarkable adsorption behavior toward hydrophobic pesticides and emerging pollutants because they allow surface customization and controlled swelling behavior. The selection of methodology shapes pore dimensions and surface molecular charges alongside accessible functional groups because these characteristics determine both pollutant removal performance and sustainable recovery capacity of the water treatment systems. Therefore, method optimization plays a critical role in designing specific environmental remediation approaches.

2.3.3. Nanohydrogel cross-linking techniques

Nanohydrogel formulation is initiated by the interaction between polymers through covalent and non-covalent bonds by cross-linking agents, which gives stability and influences the swelling behaviour of nanohydrogel (S. Yang et al. 2023). Cross-linking agents are chemical, physical, enzymatic, and ionic, which affect the swelling behavior and hydration of the network structure through osmotic pressure difference (Maiti, Maji, and Yadav 2024). Green technology-assisted formulation techniques of nanohydrogel involve non-thermal treatments such as ultrasonication, enzymatic-assisted, microwave-assisted techniques etc, which help in forming gel structure at a faster and efficient rate than conventional techniques (Florowska et al. 2023). As compared to the traditional methods of nanohydrogel formulation, non-conventional method offers mild conditions in terms of heat and chemicals; the uniform heating helps in setup of gel network more effectively than heating in case of the conventional process. There are various techniques for enhancing starch properties, such high high-pressure processing, which changes the physical properties of starch, including gelatinization, without toxic use of toxic chemicals for enhancing nanohydrogel stability and mechanical properties (Koshenaj and Ferrari 2024). One such green-based technique is defined by (Sneh Punia Bangar et al. 2023) helps starch in increasing the surface area of starch by the reduction of starch granules and inducing active sites for chemical reactions through shear, friction, and collision, termed as ball-milling. A combination of Pulsed electric field processing with succinylation modifications produces starches with a higher degree of substitution and better thermal stability by maintaining starch molecular structure (Suri and Singh 2023). Starch functionalization occurs with controlled results by environmentally friendly solvents composed of ionic liquids and supercritical CO_2, where these solvents help in selective derivative reactions and graft processes to improve nanohydrogel properties, particularly biodegradability and reusability, along with chemical stability (Fan and Picchioni 2020). Hence, these methods are environmentally friendly and improve the performance of starch-based nanohydrogels for overcoming low stability, degradability, and limited scalability of the material.

2.4. Cross-Linking

The cross-linking process includes covalent bonding between different polymer segments from chains of polymers and they are formed either during the synthesis of polymer or during final macromolecules joining at the time of reaction (Dudeja et al. 2023). A cross-linking agent is required to complete the cross-linking mechanism which helps in enhancing the mechanical and physical properties of the polymer (Y. He et al. 2024; Deng et al. 2025). Green technology-assisted cross-linking involves various methods such as physical, chemical, and ionic cross-linking which includes microwave-assisted, hydrothermal, ultrasonication, enzymatic, and supercritical-assisted cross-linking (Lin et al. 2023). Interactions of polymers crosslinked primarily through ions interaction along with nanohydrogel lead towards weak non-covalent interactions such as hydrogen bonding, electrostatic interactions, and hydrophobic interaction (Sarmah et al. 2023). Thus, cross-linking technology for starch-based nanohydrogel formation plays an important role in enhancing molecular interaction, improving gel stability, and making biocompatible material for pollutant adsorption.

2.4.1. Ultrasonication assisted Cross-Linking

The ultrasonication technique involves utilization of high-frequency sound waves by which cavitation bubbles generate, grow, and disintegrate starch granules into smaller parts. The polymer network that forms the basis of nanohydrogel is formed by the sonication process when starch undergoes partial gelatinization and cross-linkages with chemical agents resulting in the change of morphology forming a three-dimensional network of nanohydrogel (B. Cai et al. 2022). In a study, shown in Table 2 (M. He et al. 2025) upon ultrasonication cross-linking, hydrophilicity and lipophilicity of starch increase which helps in the disruption of granules, and helps in retaining water and oil through capillary action. According to a study by Halder et al. (Halder et al. 2024), dual-synergistic physically crosslinked gelatine nanohydrogel preparation was done by using a one-pot solvent method, producing high mechanical strength whereas de-hydrothermal treatment includes condensation reaction between the anime group and carboxyl group forming crosslinks at 100-120°C exposed scaffold under high vacuum improves the structure stability, control porosity, and cost-effectiveness. Another example of waxy maize by Maet al. (Ma et al. 2022) shown in Table 2, ultrasonic-assisted crosslinking with sodium tri-metaphosphate as a crosslinking agent helps in water retention, such similar study was done on rice where the crosslinking agent was also sodium tri-metaphosphate, it increases amylose content, reversible in nature, and improves stability. Hence, the formation of a dense polymer network by ultrasonication method along with a cross-linking agent helps in efficient pollutant removal from water.

2.4.2. Natural Acids Cross-Linking

Another method is chemical crosslinking which involves chemical bonds that connect polymer backbones effectively to improve the mechanical and chemical stability of anion stability membrane by crosslinkers for the formation of a gel network (Zhilang Xu et al. 2022). It involves covalent bonds in 3D stable hydrogels, and these include enzymatic polymerization, radiation polymerization crosslinking, and co-polymerization crosslinking, a natural crosslinking agent such as citric acid, boric acid, etc. can be used for a cross-linking agent for hydrogels formation (Arayaphan et al. 2021). Most used crosslinking agents such as citric acid have three carboxyls and a hydroxyl group for cross-linking under heat and oxidized sucrose which undergoes oxidation and produces polymeric aldehyde (Y. Wang et al. 2023). As shown in Table 2, in cassava starch crosslinking agents due to its good reaction efficiency and low toxicity, the citric acid cross-linking agent has a greater tensile strength of 3.75MPa whereas oxidized sucrose provided elongation properties and maximum swelling value (Kayati, Purnomo, and Kusumastuti 2024; Sumardiono et al. 2024). Overall, natural acids promote intermolecular hydrogen bonding, electrostatic interactions, and interaction of carbonyl group with amine leading to assembly in the nanohydrogel network for pollutant adsorption from water.

2.4.3. Ionic Cross-Linking

Also, ionic crosslinking is one more method that includes the linking of polymers through ionic interactions involving oppositely charged molecules or ions which helps in bridging between chains of polymers for stabilizing the structure formed, commonly used in the formation of hydrogels (Gopinath et al. 2022). In this method, the ions are used in the form of salts chelate or an ortho ester because, in the form of metal ions, the polymers start to gel prematurely (Geng et al. 2021). Alginate is the most used material in ionic crosslinking, composed of natural hydrophilic polysaccharide, consisting of guluronic acid and mannuronic acid is nontoxic, biodegradable, and can control the mechanical properties of hydrogels based on the concentration of crosslinking ions used (Łabowska et al. 2023). As shown In Table 2 jackfruit starch is cross-linked with calcium ions helps in the formation of nanohydrogel for controlled adsorption and release of pollutants (Mokhena et al. 2024). In a study done by Fernandes et al. (da Silva Fernandes et al. 2019) hydrogels crosslinked with Mn^{2+} uptake a larger equilibrium in comparison with zinc and calcium ions. It was also observed that with an increased density of crosslinking, the diffusion constant value increased. Thus, ionic cross-linking helps in the formation of a three-dimensional network of nanohydrogel by the interaction between charged starch molecules and metal ions, helping in retaining water.

2.4.4. Microwave-assisted cross-linking

Microwave radiation generates a heating effect which increases the interaction of polar molecular attraction, the dipolar rotation mechanism leading towards localized heating, and disruption of hydrogen bonding, promoting cross-linking for nanohydrogel formation (Albarqi et al. 2022). In Table 2, microwave-assisted crosslinking of rice starch shows the improvement in the adsorbent material functionality, water within the starch matrix crystallizes and freezes thawing creates a porous network (P. Li et al. 2024). Thus, microwave-assisted technology helps in breaking the phosphate ester bond in crosslinking agents helping the phosphate anion to react with the hydroxyl group of starch molecules which helps in strengthening the gel network of nanohydrogel and provides stability.

2.4.5. Enzymatic assisted cross-linking

Enzymatic treatment involves the use of various enzymes such as amylase, cellulase, etc. which break hydrolysis of the starch molecule in simpler units, and crosslinkers help contain functional groups of polysaccharides and form covalent bonds (Mora and Toldrá 2023). This crosslinking helps in the formation of a network for nanohydrogel due to increase in the hydrophilic nature for the adsorption of pollutants. As shown in Table 2 (Montes et al. 2022) corn starch enzymatically hydrolyzed by α-amylase and cross-linked by sodium alginate which leached out amylose from starch, a stronger gel structure was seen when the hydrophilic nature of alginate was high. Another study was done on potato starch by Fang et al. (F. Fang et al. 2020), shown in Table 2, explains that ionic crosslinking involves the electrostatic interaction between gel structure and starch that affects the nanohydrogel structure. Thus, enzymatic-assisted crosslinking of starch helps in the formation of a network for the adsorption of pollutants from water through an environmentally friendly approach.

2.4.6. Photo-cross-linking

Photo-cross-linking uses UV or visible light as a cross-linking agent for forming a three-dimensional nanohydrogel network by introducing photo-reactive groups such as acrylate, vinyl group, or methacrylate in the presence of photo initiators such as benzophenone. It is a non-toxic method of cross-linking to promote environmental compatibility of starch-based nanohydrogels and majorly impacts the mechanical strength, increased adsorption capacity, and swelling behavior of starch. A study on Yuca starch as given in Table 2, upon reacting with sodium hydroxide and cinnamyl chloride was used for cross-linking between starch-cinnamyl ethers by [2+2] cycloaddition reaction of cinnamyl moieties upon UV irradiation at 254nm, which is water soluble and effectively used for various uses such as pollutant removal, packaging, and film formation (Petroni et al. 2025). Another study (Zain, Wahab, and Ismail 2018) on cassava starch was conducted using sodium benzoate as a photo-initiator for photo-crosslinking, which involved irradiation for different times ranging from 30 to 480 minutes at wavelengths above 290nm. The study concluded that the effect of photo-crosslinking increases by increasing temperature up to 120 min., and it helps in improving gelation due to increased carboxylate formation and increased tensile strength.

2.4.7. Novel cross-linker

A study was done on starch modification by cross-linking with microgel latex prepared from inverse emulsion polymerization, for the preparation of starch-based nanohydrogel, which increased the nanohydrogel capacity for absorption and high gel strength at 120 °C and reaction time of 100min. at higher adsorbence at 17g/g compared to commercial adsorbent (Amiri et al. 2019; Ibrahim, Abd-Eladl, and Abou-Baker 2015). A study done on using cross-linkers such as Allyl sorbitol, Allyl mannitol, and Allyl pentaerythritol for the synthesis of starch-based nanohydrogel for the removal of heavy metals resulted in the removal of copper metal ion by 81.8,81.6 and 86.8%, whereas nickel metal ion removal at the rate of 96.8,96.9,99.0% by three different cross-linkers (A. Mishra et al. 2021). For comparing novel cross-linking of corn starch by citric, lactic and malic acid and micro N_2 bubble assisted esterification, it was found that modified starches resulted in lower crystallinity and a higher degree of substitution in micro N_2 bubble assisted esterification at 0.32 to 0.49% whereas unmodified esterified starches had 0.24 to 0.37% of reduced crystallisation. However, no correlation between the organic acids and with degree of esterification was observed, and a reduction in digestibility of micro-nitrogen esterification starches was observed upon comparison to native starch due to its cross-linked and compact microstructure (Abedi et al. 2025). Thus, novel cross-linking methods are effective for modifying starch-based nanohydrogels.

Table 2- Crosslinking techniques of starch

2.5. Application of starch-based nanohydrogels as an adsorbent

Starch-based nanohydrogels as adsorbents are useful due to their large surface area and more availability of polar sites by modification which produces specific functional groups such as hydroxyl, phosphate, amino, and ester groups on the backbone of starch (Haq et al. 2022). Addition of functional groups attracts pollutants on these active sites through electrostatic forces, hydrogen bonding or ionic exchange interactions, for instance Pb^{2+}, Cd^{2+} and Cu^{2+}, are heavy metal cations that are adsorbed through electrostatic interactions and ion exchange due to carboxyl and sulfonic groups create a negative surface charge (X. Yang et al. 2019). Crosslinking of starch with other materials helps in increasing the biocompatibility and porosity and is efficient in removing a variety of contaminants such as dyes, heavy metals, antibiotics, pesticides, and organic pollutants that damage the human as well as aquatic life (Hugar et al. 2024). According to a study by Costa et al. (T. B. Costa et al. 2024) studied, adsorption capacity depends upon various factors for instance pH, contact time, reactive sites, concentration of pollutants, and charges on the surface of starch-based adsorbents. A study done on the causes of pH levels on the nature and working of nanohydrogels, it showed that the functional groups of acidic and basic nanohydrogels can be ionized easily and releases protons when they get attached to the polymer backbone (Ahmadi et al. 2024).In a nanohydrogel hydrophilic surface imparts OH and COOH groups, increases high amount of water absorption that enhances the penetration of contaminants inside the nanohydrogel structure at high pressure or temperature , and expands the polymer network for binding of pollutants(H. Liu et al. 2024). Thus, starch-based nanohydrogel material is highly effective for pollutant removal from water through nanoscale porosity, and large surface area which helps in maximizing adsorption sites whereas conventional adsorbents are non-biodegradable, have fewer binding sites for formation and adsorption of pollutants on the surface.

2.5.1. Removal of Antibiotics

For the adsorption of antibiotics, pH, adsorbent dose and concentration of antibiotics is effective as they affect the solubility, and increase the adsorption efficiency due to the large surface area and interaction between adsorbents (Anuar et al. 2023).In Figure 3A, it is shown that fluvastatin showed absorption efficiency at 782.05 mg g^{-1} for antibiotics through hydrophobic interaction where molecules of antibiotics are trapped onto porous sites, but it has a hazardous effect on the respiratory tract, green and environmentally friendly carboxymethyl starch complex had better performance when compared to corn starch (G. Sharma et al. 2017; Miao et al. 2022) shown in Table 3. Starch-based nanohydrogel helped in the adsorption of Diclofenac by 109.28mgg^{-1}, ofloxacin by 120.99 mgg^{-1}, fleroxacin by 425.50 mgg^{-1}, levofloxacin by 87.34 mgg^{-1}, ibuprofen by 70.0 mgg^{-}1, ciprofloxacin by 190.0 mgg^{-1} shown in Table 3, (Mohamed and Mahmoud 2020). In Table 3, according to Fang et al. (K. Fang et al. 2021) Doxorubicin hydrochloride adsorption efficiency reached 85.46% by carboxymethyl cassava starch functionalized magnetic nanoparticles. Starch-based adsorbent helped in the removal of tetracycline by 8.79 mgg^{-1} (X. Zhang et al. 2023), which was less as compared to magnetic starch polyurethane polymer which showed 19.272 mgg^{-1} capacity, but the highest adsorption was seen in the case of carboxymethyl-starch based grafted magnetic bentonite at 169.7 mgg^{-1}(Rong et al. 2024). So, nanohydrogels have the potential for the efficient removal of antibiotics from aqueous systems, offering a sustainable and innovative solution to address environmental contamination and water purification.

2.5.2. Removal of Dyes

According to the studies done by Manjunatha et al. (Manjunatha et al. 2024; Dang et al. 2022) shown in Table 3 ,proved that the best method to eliminate methylene blue dye by 2967.66mgg^{-1} is through pretreatment of starch nanohydrogel by sodium hydroxide also, in Figure 3B, it is seen that dye molecule easily penetrate and attach onto active sites of adsorbent material, compared to other nanohydrogel formation which is lowest at 64.05mgg^{-1}, whereas phosphorylated starch adsorbs 1036mgg^{-1} methylene blue which explains that radiation-induced hydrogels are less effective for elimination of MB dye and maximum absorbance capacity of methylene orange dye was 1107.2mgg^{-1} by corn starch based nanohydrogel and minimum at 238.1mgg^{-1} as shown in Table 3. Upon increasing the cassava starch content, the adsorption and removal capacity of methylene blue dye was minimum at 0%wt dosage and pH 2 i.e. 73.5% and maximum at 50 wt.% of cassava starch dosage at 91.7% and pH 4 due to the availability of active sites which increases the absorption of dye molecule (Arayaphan et al. 2021). As shown in Table 3 by (Majeed et al. 2024) explained that starch-based nanohydrogel showed 2500mgg^{-1} crystal violet adsorption capacity, Eosin Y had 143mgg^{-1} adsorption capacity by electrostatic interaction of nanohydrogel. Hence, efficient dye removal from water is essential for safeguarding water quality and promoting sustainable environmental practices.

2.5.3. Removal of Heavy metals

For the adsorption of Cu (II) ions, in Figure 3D, it is shown that the ions present on the adsorption material are replaced through ionic interaction as well as covalent bonding, which according to Hou et al. (Hou et al. 2024) had maximum 94.15mgg^{-1} adsorptive capacity compared to adsorption capacity of 75.5mgg^{-1} through starch grafted and cross-linked with Fe_3O_4-g-p (AA-r-HEMA) shown in Table 3. According to (Haq et al. 2022; Duquette and Dumont 2018), also shown in Table 3, for maximum adsorption of Cd^{2+} ions at 63.46%, a nanohydrogel with 12.5% synthesized laponite RD crosslinked with starch was used, by using starch poly nano-composite nanohydrogel material Cu^{2+} and Pb^{2+} ions were removed at 95.4% and 88.4% respectively whereas by using nanohydrogel graft polymerized onto normal, high and waxy starch using acrylamide as a cross-linker to remove Cu^{2+}, maximum adsorption at 1.36 mmol/g and waxy corn had minimum ratio at 1.28mmol/g Haq et al. (Haq et al. 2022) explained in Table 3. Overall, heavy metals removal is essential for water pollutants removal for consumption by the adsorption method.

2.5.4. Removal of Pesticides and Herbicides

According to Chen et al. (X. Chen et al. 2021), shown in Table 3C, herbicides like prometryn and atrazine were removed using maize starch bio adsorbent treated with glucoamylase and amylase which resulted in increasing the surface area and removal efficiency compared to the natural degradation by 61%. In Figure 4D, fenamiphos and bromacil form hydrogen bonds between hydrogen donors present on adsorbent material and acceptors present in the pesticide molecule (S. G. Costa et al. 2024). The interaction with the nonpolar site of starch resulted in the highest adsorption than others, also porous β-cyclodextrin polymer showed efficient uptake than nonporous cyclodextrin in the case of metolachlor (X. Huang et al. 2024; Shraftar and Ghaemi 2024). In a study by Dehghani et al. (Dehghani et al. 2024), chemisorption between starch and oxadiazon removed 90% of oxadiazon herbicide from aqueous solutions as well and atrazine was removed at adsorption capacity of 17.92mg g^{-1} as given in Table 2 (do Nascimento et al. 2022), by aqueous solution by starch modified sepiolite and starch-based mesoporous activated carbon had adsorption capacity for pyraclostrobin at 66,2mgg^{-1}. Hence, efficient removal of pesticides and herbicides from water reduces harmful pesticide and herbicide concentrations through an adsorbent mechanism for improving the quality of water.

Fig 2- Application of pollutant removal from starch

2.5.5. Removal of Microplastic

Starch-based nanohydrogels are efficient in eliminating microplastics from water bodies as defined by (Fu et al. 2023) gelatine and corn starch crosslinking create a biodegradable substance, and freeze-drying methods enable the development of an ultralight sponge that can effectively remove 90% of microplastics at its peak operational conditions. The sponge resulted in the removal of 60–70% of materials when tap water, seawater samples, and soil containing surfactants were tested (M. Sun et al. 2020; Zhou et al. 2021).The sponge becomes glucose when treated with enzymes after use, resulting in environmentally sustainable practices and minimizing the secondary pollutants. The use of St-CTA starch-based coagulant together with polysilicic acid creates an effective and synergistic method to eliminate nano- and micro-sized plastics like polystyrene and polyvinyl chloride from different water bodies where St-CTA neutralizes charges by PSA forming compact structures which can be effectively filtered from water (Hu et al. 2022). Polyoxometalate nanocluster-infused triple IPN hydrogels introduce microplastic degradation by using UV light by exhibiting high absorbance of 95 % for polyvinyl chloride and 93 % for polypropylene at pH 6.5 with reusability up to 5 cycles (Dutta, Misra, and Bose 2024). The ability to both adsorb along degrade microplastics while using UV light makes this technology an eco-friendly substitute for current filtration approaches.

Pollutant	**Adsorbent material**	**Maximum Adsorption capacity**	**Key Features**	**References**
Antibiotics (Fluvastatin)	Starch-based magnetic hydrogel (NFe3O4@Zn (GA)/starch)	782.05 mg/g, 68.07% at 55°C	Highest adsorption, exceptional regeneration ability after 5 cycles	(Saracoglu and Ozmen 2021)
Diclofenac, ofloxacin, fleroxacin, tetracycline, levofloxacin,	Starch based nanohydrogel	425.50 mg/g in case of fleroxacin	Lower adsorption compared to other materials	(Sarmah et al. 2023)

Ibuprofen, ciprofloxacin			(in case of tetracycline)	
Doxorubicin hydrochloride	Carboxymethyl cassava starch functionalized magnetic nanoparticles	85.46%		(Sellami et al. 2021)
Tetracycline	Carboxymethyl-starch-based grafted magnetic bentonite	169.7mg/g	Highest adsorption capacity	(Shang et al. 2024)
Penicillin	Carboxymethyl starch complex	Better performance than corn starch nanohydrogel	Better performance than corn starch nanohydrogel	(Thuppahige et al. 2023)
Pesticides and Herbicides (Prometryn, Atrazine**)**	Maize starch bio adsorbent treated with glucoamylase and amylase	61% increase in removal efficiency compared to natural degradation	Increased surface area and removal efficiency.	(Torres and De-la-Torre 2022)
Bromacil, Fenamiphos, Bromaci, Benalaxyl, Butachlor	Cyclodextrins obtained from enzymatic extraction of starch	Highest adsorption for Fenamiphos and Bromacil	Porous β-cyclodextrin polymer showed better adsorption than non-porous cyclodextrin in metolachlor.	
Oxadiazon, Atrazine	Starch modified sepiolite	90% removal of Oxadiazon, 17.92 mg/g for Atrazine	Chemisorption between starch and oxadiazon	(C. Sun et al. 2024)

Dye (Methylene blue)	Phosphorylated Starch	1036 mg/g	-	(G. Sharma et al. 2017)
	Other Nanohydrogel Formation	64.05mg/g	Lowest adsorption capacity for MB dye	
	Cassava Starch-based Nanohydrogel	91.7% removal	50 wt.% dosage, pH 4, maximum removal, 0 wt.% dosages, pH 2, minimum removal	
Crystal Violet	Starch-based Nanohydrogel	2500 mg/g	-	(Majeed et al. 2024)
Eosin Y	Starch-based Nanohydrogel	143mg/g	Electrostatic interaction	
Heavy metals Cd (II)	Hydrogel with 12.5% synthesized laponite RD cross-linked with starch	63.46%	-	(Duquette and Dumont 2018)
Pb (II)	Starch poly-nanocomposite hydrogel	95.4%	-	(Raj et al. 2023)
Cu (II)	Nanohydrogel graft polymerized onto normal starch using acrylamide	1.36mmol/g	At 5 pH	

Microplastic PVC, Polystyrene, Polypropylene	Gelatin + Corn starch, Polyoxometalate Nanocluster-infused Triple Interpenetrating Network (IPN) nanonydrogel and tarch-based Cationic Tannin Amine) + Polysilicic Acid (PSA) Coagulant	Up to 90%, 60–70% in tap/seawater/soil, 95% (PVC), 93% (PP)	Biodegradable; ultralight structure; effective in complex matrices; enzymatic conversion to glucose reduces secondary waste, High UV absorption; eco-friendly degradation; reusable for up to 5 cycles; works effectively at pH 6.5	(Tan et al. 2009)

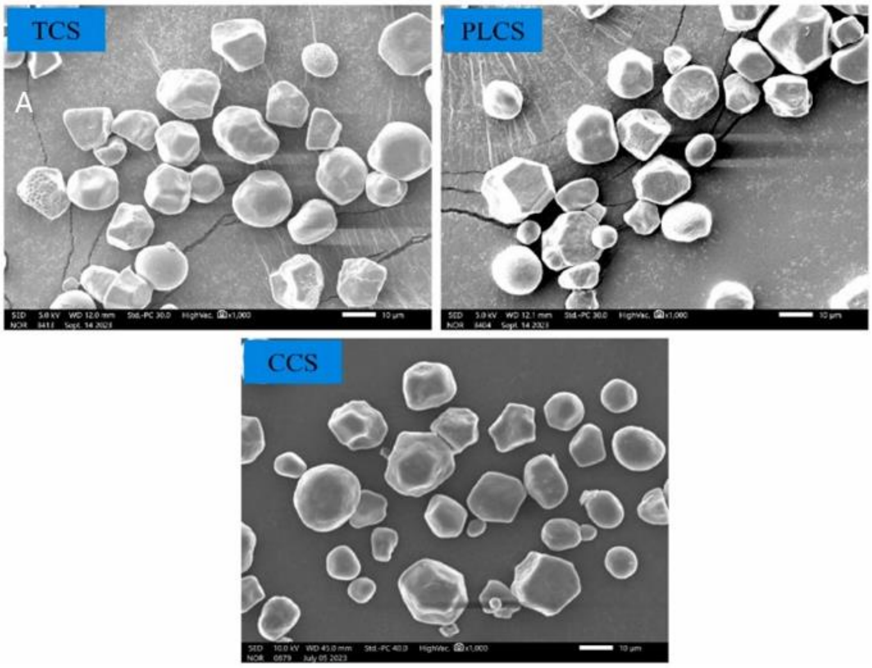
Table 3- Removal of Various Pollutants by starch based nanohydroge

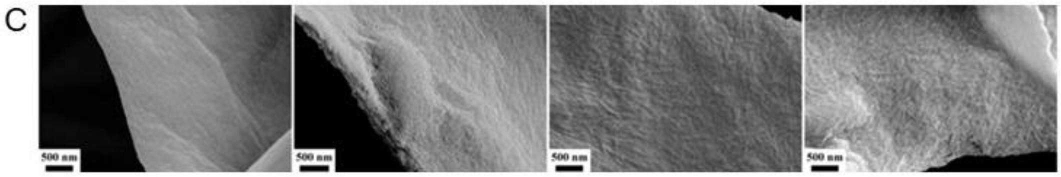
2.6. Characterization of starch-based nanohydrogels

Characterization is an essential process that helps in determining and understanding the physical, chemical, structural, and functional properties of a material which helps in the assessment of certain parameters such as amylose content, amylopectin content, pasting properties, granular size, gelatinization behavior, and molecular weight distribution in case of starch (Zambelli and Mendonça 2024). For example, to determine the crystalline defects, orientation, and defect of a material, the X-ray diffraction technique is used which includes the production of the monochromatic electron beam, whereas to determine the surface chemistry of a material Scanning electron microscopy is used (Herrero, Camas, and Ullah 2023) . Hence, several other techniques including Fourier Transform Infrared Spectroscopy **(**FTIR), Nuclear Magnetic Resonance, Transmission Electron Microscope (TEM), and rheological analysis, provide crucial insights into their structural, morphological, and functional properties, facilitating the optimization of these materials

2.6.1. Scanning electron microscopy (SEM)

Scanning electron microscopy is a surface-imaging technique that helps to produce images of a sample by directing a focused beam of electrons, for conducting morphology studies related to the size, shape, purity, and polydispersity of the specimen. It is considered a powerful technique with magnification power in various ranges and acceleration voltage of 30kV focused on the sample using a dense electron beam which converts the signal and displays it on the screen (Xiao et al. 2022). By comparing various studies of (Thuppahige et al. 2023; Zeyi Liu et al. 2024) it was observed that the largest granule size range was seen in the case of both peeled and unpeeled banana-resistant starch i.e. 13.1 – 59.6 µm and 30.99µm was the smallest granule size observed in case of corn starch as shown in Figure 3 A. Most of the starches were flat, spherical, irregular oval, and elongated in shape and unpeeled bananas contained some fibrous structure due to impurities and other compounds present in the granules (Miah et al. 2023). A porous Surface morphology was shown by the SEM images due to crosslinking agent and the grafted structure of polymer which helps the nanohydrogel structure to take up water for providing greater contact with the surface (Zhu et al. 2015). As shown in Figure 4 C, Cellulose nanocomposite material as a cross-linking agent the higher swelling ratio of nanohydrogels can be achieved by using less CNC, but can affect the structure and functional groups if used in greater amounts (P. He et al. 2022). Overall, SEM serves as a vital analytical tool for providing high-resolution imaging and surface morphology insights, thereby significantly contributing to the understanding of material characteristics and structural details.

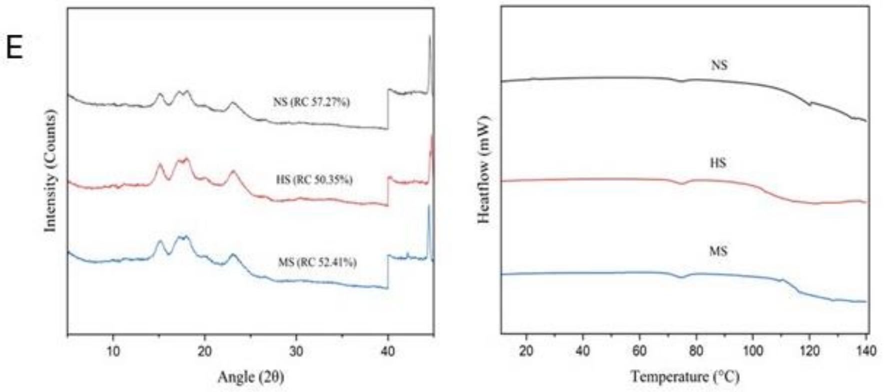


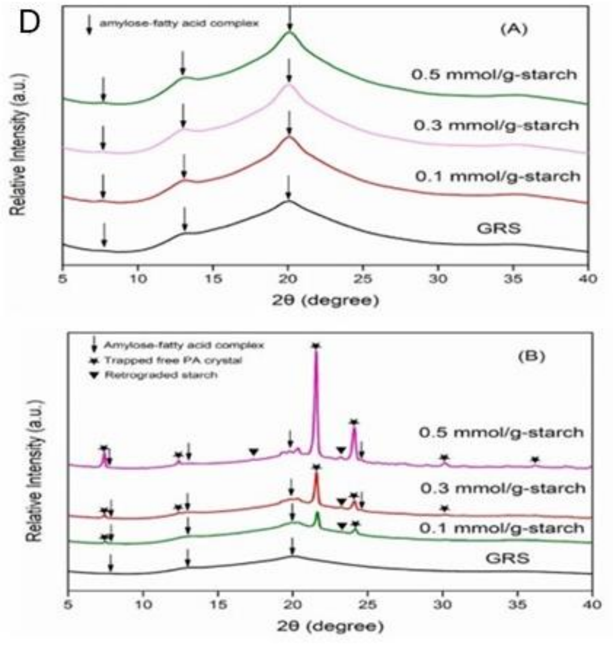


2.6.2. X-ray diffraction (XRD)

X-ray diffraction is an analytical technique which is based on the principle of Bragg's law which states that when the x-ray is incident onto the surface of the crystal, (θ) angle of incidence equals the angle of reflection and without losing photon energy leading to constructive interference when path difference is equal to the whole number of (λ)wavelength. It is a technique that gives information about the structure, texture, degree of crystallinity, tension, and defects, also it talks about the arrangement of atoms, dimensions of lattice, bulk and non-bulk material structure(Gond et al. 2022; P. Kumari et al. 2024) . In some studies, the effect of microwave treatment resulted in millet starch having maximum crystallinity of about 57.27% due to large crystallite size showing sharp and defined diffraction peaks whereas filed pea starch resulted in minimum crystallinity at 5.15% due to gelatinization of starch at high temperature (M. Cai et al. 2024; Balakumaran et al. 2023). According to Guo et al. (Guo et al. 2023), as shown in Figure 3B, pea starch showed the highest peak at 22.95 indicating C-type crystalline structure and B-type structure peaked at 17°, however, starch retrogradation was indicated by two B-type diffraction peaks at 17° and 23° respectively, also gelatinized rice starch showed the lowest relative crystallinity at 8.46% and highest was at 35.15% in case of rice starch complexed with lauric acid.

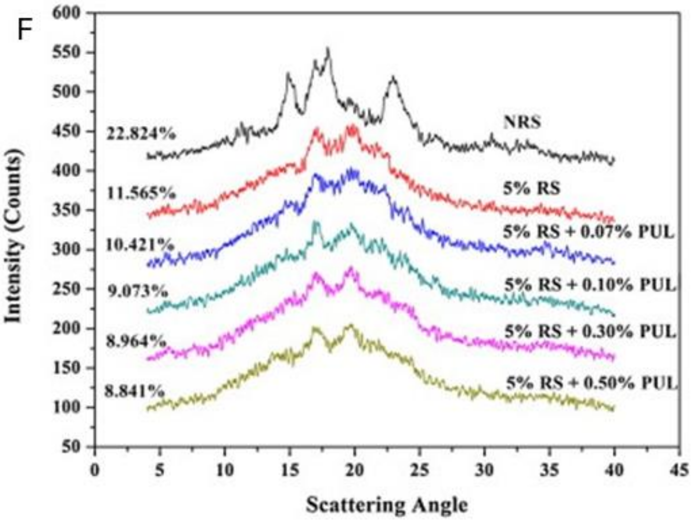
The peak observed at $2\theta = 17.0°$ reveals a semi-crystalline structure of starch nanohydrogel shown in Figure 1 b, whereas at peak $2\theta = 22°$ nanohydrogel showed an amorphous structure, which shows the presence of a crosslinking agent with the starch nanohydrogel formation (Olad, Doustdar, and Gharekhani 2020). At 20°, a peak was observed for pea and corn starch nanohydrogels which showed amylose-lipid complex formation, and it also indicates B+V crystallinity type pattern at reflections of 2θ=7.6°, 13.2°, 17.0°, and 20.0 and proved that the crystallinity increases with increasing of storage time as shown in Figure 4D (L. Chen et al. 2015). In as study, it was observed that V-type amylose crystallites were seen in the case of normal maize starch nanohydrogel on hydrothermal treatment whereas no detachable long-rage ordering feature was seen in low amylose containing starches producing hydrogels (Yanqi Liu, Xie, and Shi 2016). The high amylose maize starch nanohydrogels having B-type structure and high degree of ordering showed no change due to hydrothermal treatment during nanohydrogel formation, these differences are also due to the different retrogradation rate of amylose and amylopectin as shown in figure F and G (Raigond, Ezekiel, and Raigond 2015; Koev et al. 2020). X-ray diffraction provides critical insights into the crystalline structure and molecular arrangement of nanohydrogels, serving as a key analytical tool to optimize their design and functionality for advanced applications.





2.6.3. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy is regarded as the most used chemical analysis technique which helps in determining the various types of functional groups, molecular vibrations, and molecular interactions between complexes, it is determined by identifying the bending and stretching of chemical bonds through absorption of energy with the help of infrared spectroscopy. The infrared spectrum generates various frequencies which are converted into light intensities through interferogram (Hanan et al. 2024; Nirmala and Vijayashreenayak 2011). In a study done on potato starch and millet as shown in Figure 3H, by Jain et al. (Z. Zhang et al. 2024) peak intensity increased due to an increase in hydrogen bonds in starch molecules, peaks are observed due to various factors such as bending vibrations of C-H bonds, between hydrogen bonds, and antisymmetric stretching vibrations were also seen between C-H_2 and C-H bonds in respective ranges. In the case of potato starch-based nanohydrogel, the stretching vibrations of C-O-C bonds were proven by three peaks at 980, 1085, and 1165 cm^{-1}; however, peaks at 3430 and 2930 cm^{-1} showed stretching vibrations of OH and CH groups(Olad, Doustdar, and Gharekhani 2020). Grafting of lowest ratio. Also, starch nanohydrogel due to the hydrogen-bonded -OH group showed peak at 3,285.8 cm−1, whereas monomers onto the starch chains is seen by the nanohydrogel being shifted to the lower wavelengths at 1150 and 1350 cm^{-1} (Rashidzadeh et al. 2014). A study done on pea, potato, and corn starch for nanohydrogel formulation, in Figure 4 I, among these starches, pea starch nanohydrogel showed the highest ratio of 1,047/1,022, whereas potato starch showed the CH_2 asymmetric stretching of CH_2OH groups of starch showed peak around 2,900 cm^{-1} (Azman et al. 2016) . Hence, FTIR analyzes structural modifications in adsorbents and has the potential for efficient water purification and contaminant removal.



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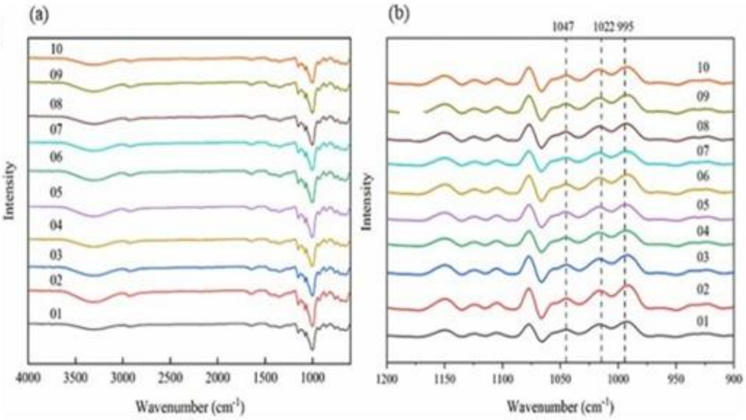
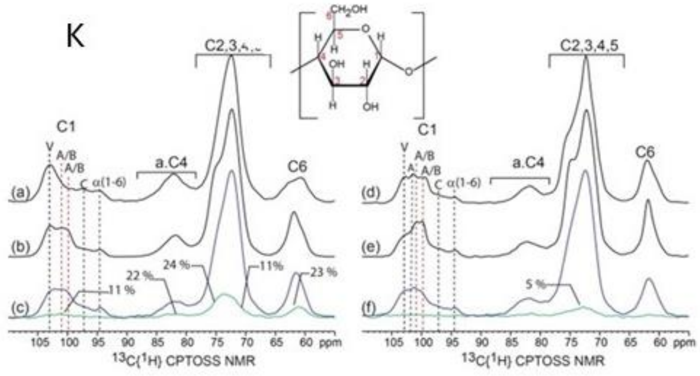


2.6.4. Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance spectroscopy is a technique that is used for determining molecular structure, content, physical and chemical properties, and purity of atoms and molecules by using a magnetic field, based on the spins of atomic nuclei specially hydrogen and carbon, it uses radio-frequency electromagnetic radiation to interact with nuclei of atom immersed in a strong magnetic field (Anwar, Amin, and Hoque 2023). According to Yong et al. (Yong et al. 2018) purple sweet potato starch's characterization as shown in Figure 4K detected three types of crystallinities which refers to the order of solid material such as polymers, fibers, or semi-crystalline substances, in which type A had the most number of peaks, the highest being at 102ppm and lowest at 100ppm and resistant purple starch also had three peaks which came under A-crystallinity, and B-type had two peaks lowest at 100ppm and highest at 101ppm. While comparing corn, potato, and yeast-derived starches, a study by Russell et al. (Russell et al. 2024) peaks of B at the C1 position overlap with two of type A signals, while the C1 signal is at 103ppm, others from C1 to C6 had sites yield signals at different ppm. Among all starches, amylose has the most disordered structure at 103ppm while the least disordered structure was of potato starch. Difference among five varieties of maize starch nanohydrogels is revealed in the 100-104 ppm shoulder region, which gives information about the V-type amylose crystalline arrangements and amorphous region combinedly, where Hi-maize showed higher amylose content than waxy maize(B. Zhang et al. 2014). In figure 4 L, three peaks were seen within the C-1 environment (99.0-101.8ppm) where central peak exhibited more local mobility at 100.3ppm when compared to side peaks at 99.8 and 100.8ppm (Koev et al. 2020). Thus, NMR spectroscopy serves as a powerful technique for elucidating the molecular dynamics, structural interactions, and functional modifications of nanohydrogels, contributing significantly to their development for targeted applications.



2.6.5. Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy is a powerful surface characterization and analytical technique that uses a beam of high energy electron through a material for imaging its internal structure at a high magnification and resolution, typical accelerating voltage range from 80kV-300kV (Raj et al. 2023). As the electrons interact with the sample, some are scattered while others pass through, depending on the material's density and composition, detectors capture the transmitted electrons, creating a highly detailed image at the atomic or molecular level(Micheletti and Shah 2024). This technique allows for exceptional resolution, making it possible to observe fine details such as atomic arrangements, crystallographic structures, and nanomaterials with clarity far beyond what is achievable with traditional light microscopy (Majithia and Barretto 2023). According to Sharma et al. (G. Sharma et al. 2018) in a study about starch as shown in Figure 4 M, evaluated that the solubility parameters did not change in the tested temperature range without any chemical or physical reaction. A study between pea, potato and corn starch- nanohydrogel formation, which showed that the compact and well-distributed spherical structure after 12h of retrogradation was seen in the case of pea starch among the three starches due to lower amylose content of other starches, which ensured that retrogradation during storage contributes to changes in the morphological structure of starch in figure 4 N (Q. J. Sun et al. 2014; Ji et al. 2018). It was observed due to the low modulus of the material, so in the form of the magnitude of the complex module, more reliable modulus information was evaluated through interdisciplinary research and technological innovation. Starch-based nanohydrogels are poised to play a critical role in the advancement of next-generation water treatment systems and contribute meaningfully to global efforts in ensuring clean and

safe water access.

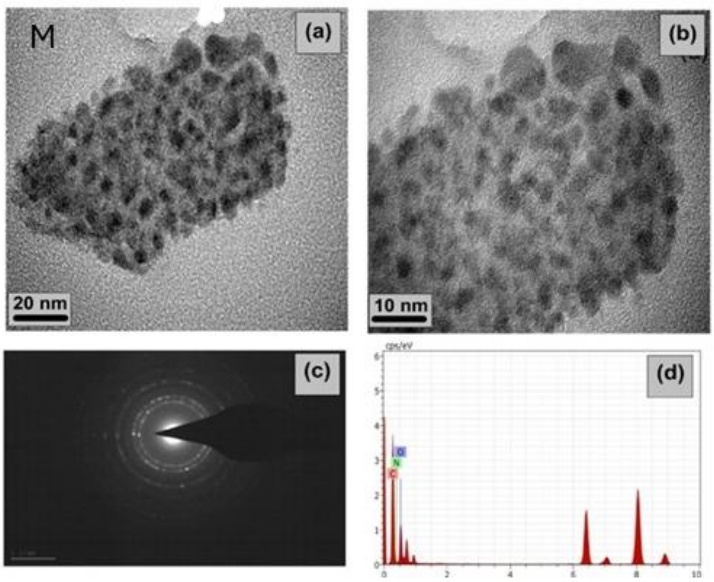


Fig. 3- Characterization of Starch (A) Scanning electron microscope micrograph (B) Scanning electron microscopy micrographs depicting surface morphology of extracted peel starch (a), Extracted bagasse starch (b) and commercial cassava starch (c) observed under 1000× and 2000× magnification levels, (C) The influence of nanocellulose on hydrogel properties and drug delivery performance. a) SEM images of the cross-section of CNC-reinforced quaternate cellulose hydrogel (0 wt% CNC (a_1), 1 wt% CNC (a_2), 1.5 wt% CNC (a_3), 2.5 wt% CNC (a_4)), (D) X-ray diffraction patterns of (A) GRS and gelatinized starch–LA mixtures, and (B) gelatinized starch–PA mixtures, (E) Diffractogram of native and modified white finger millet (WFM) starch; Thermogram of native and modified white finger millet (WFM) starch, (F) X-ray diffraction patterns of native rice starch and gelatinized RS pasta containing various ratios of pullulan after storage at 4 °C for 7 days.(G) X-ray diffraction patterns of different starch samples: (a)the effect of the amount of ethanol, (b) the effect of treatment temperature, and (c)the effect of treatment time, (H)FTIR spectra (a) and deconvoluted FTIR spectra (b) of foxtail millet starches, (I) FTIR spectra of starch, St-graft-poly(AA-co-AM)/PVA, St-graft-poly(AA-co-AM)/PVA/CNC, PVA, and CNC (a), and XRD pattern of CNC, St-graft-poly(AA-co-AM)/PVA, St-graft- poly(AA-co-AM)/PVA/CNC, PVA, and starch,(F) NMR spectra of (a)amylose, (b) native corn starch, (c) deuterated yeast starch, (d) High amylose corn starch, (e) native potato starch (f) non-deuterated yeast starch, (J) The FT-IR spectra of the Clin (a), NaAlg (b), NaAlg–*g*– p (AA–*co*–AAm) (c), NaAlg–*g*–p (AA–*co*–AAm)/Clin (d), and NaAlg–*g*–p (AA–*co*–AAm)/Clin/NPK fertilizer, (K) NMR spectra of (a)amylose, (b) native corn starch, (c) deuterated yeast starch, (d) High amylose corn starch, (e) native potato starch (f) non-deuterated yeast starch ,(L) 13 C NMR spectra: (A) maize and potato ghosts with freeze-drying (FD) or ethanol-drying (ED); (B) maize ghosts with freeze- or ethanol-drying; (C) digestion residues from maize and potato ghosts. Separation of 13 C NMR spectra into ordered subspectra: (D) digestion residues from maize and potato ghosts. MS, maize starch; PS, potato starch; G-180, granule ghost hydrolysis for 180 min, (M) TEM images (a,b) SAED pattern (c) EDS (d) of GA-cl-poly (AAm) NHG., (N) TEM image of starch nanohydrogels prepared with different retrogradation time (6 h, 12 h,24 h, and 36 h)

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Chapter 3

Scope of Study

3. Scope of the study

The present study explores the potential of red cowpea (*Vigna unguiculata*) starch as a sustainable and eco-friendly raw material for the development of advanced adsorbents aimed at environmental remediation. Specifically, this research focuses on the extraction, chemical modification, and characterization of red cowpea starch using maleic acid to enhance its physicochemical, functional, and structural properties. The scope encompasses evaluating how maleic acid modification improves key attributes such as thermal stability, solubility, antioxidant and anti-inflammatory activities, and resistance to enzymatic digestion, making it suitable for use in both food and non-food applications. A major thrust of the study lies in assessing the modified starch's effectiveness as an adsorbent for the removal of synthetic dyes and heavy metals from contaminated water, addressing critical issues of water pollution and public health. Additionally, the study highlights the green and sustainable aspects of using underutilized, climate-resilient crops like red cowpea to replace conventional starch sources, thereby supporting biodiversity, crop diversification, and circular bioeconomy models. Through structural, thermal, and functional characterization, the research provides insights into the suitability of maleic acid-modified red cowpea starch for applications in biodegradable packaging, wastewater treatment, and controlled drug delivery systems. Thus, the scope extends across disciplines including food science, environmental engineering, material science, and sustainable development, offering a multidisciplinary contribution to innovative biopolymer-based solutions.

Chapter 4

Objectives

CHAPTER 4

4. Objectives

- To extract and modify the red cowpea starch
- To evaluate rheology, pasting properties, and characterise the red cowpea seed starch.
- To develop red cowpea seed starch-based adsorbent for pollution removal.

Chapter 5

Materials and Methods

CHAPTER 5

5. Materials and methods

5.1. Sample collection

Red cowpea (Vigna unguiculata) was purchased from the local market of Phagwara, Jalandhar, India. The analytical grade chemicals such as Sodium hydroxide (NaOH), Maleic acid ($C_4H_4O_4$), Ethanol (C_2H_5OH), and Hydrochloric acid (HCl) were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. The use of acid-washed glassware ensured the precision and level of purity throughout the experiment procedure.

5.2 Proximate analysis of starch

A proximate analysis of red cowpea was carried out using AOAC methods. The samples at 105°C were dried for moisture content to a constant weight (Howitz & Horwitz, 2019), whereas for protein content (N × 6.25), the micro-Kjeldahl method was used (Quinton, 2002). Ash content was determined by incineration of the sample at 550 °C at 16h. Fat content determination used the Soxhlet method and a solvent (Purwandari et al., 2023). By subtracting the amount of fat, protein, and ash and residual weight, carbohydrate content was calculated.

5.3 Extraction of starch

The red cowpea starch was extracted by the alkaline steeping method outlined by Costa et al. (Costa et al., 2022) with some modifications. After washing with water to get rid of dirt present on the pulse. Red cowpea was dispersed in the solution of hydrochloric acid at 0.25% for 24h in ratio 1:2(m/v) at 4°C. After 24h, supernatant was discarded, pulse softened, and loses its outer coating was removed manually before slurry preparation by a mixer blender (Philips HL 7720, Philips, Amsterdam, Netherlands) and neutralized with 2M HCL to pH7. The acquired mixture was sieved through a 150 mesh before centrifugation at 1000 rpm for 10 minutes (REMI PR-24, REMI Ltd., Mumbai, Maharashtra, India), 5-6 times until a white coloured starch was obtained. The starch at 105°C is dried until a powder is obtained. It is ground to get an equal texture and stored in an air-tight container.

5.4 Amylose content

Amylose content was determined by the procedure followed by (Baek et al., 2019), 0.5g of starch sample was added to 95% ethanol (volume fraction, 5mL), then mixed with 45mL 1N NaOH. At 85 °C for 10min., the resultant mixture was then heated in a water bath, and lastly, 100mL of total volume of solution was made by using distilled water. From this solution, 5 mL of solution was used and mixed with 1mL acetic acid (1N acetic acid) and 2mL iodine solution, again, 100mL of solution with distilled water was prepared. Lastly, at 620nm, the absorbance was recorded, and amylose content was calculated.

5.5 Modification of starch

As per the methodology given by (Park & Park, 2025), 20g (db) of starch was mixed with 28 mL of water, with maleic acid (MA) at either 15% (15% starch basis,15MA starches). The pH was then adjusted to 5 with the use of 5 M sodium hydroxide, followed by stirring at room temperature for 60 min. The mixture was then dried at 40 °C for 16 h and milled using a cyclone mill (Twister; Retsch, Haan, Germany) with a 0.5 mm mesh sieve. Subsequently, the starch was then subjected to heat treatment at 120 °C for 12 hours. Then it was redispersed in distilled water, and the pH was adjusted to 7. The dispersion was centrifuged at 2657 ×g for 15 minutes, and lastly, the supernatant was discarded. Settled residue at the bottom was again washed thrice with water, followed by a final rinse with ethanol. The sample at 40 °C was again dried for 16 hours by using the cyclone mill.

Application of starch

5.7.3.1 First-order kinetics model

In-vitro starch digestion is calculated by first-order kinetics model majorly using single first-order kinetics model, Logarithm of slope (LOS) plot (sequential model),

Parallel kinetics (PK) model and combined parallel and sequential (CPS) model. The SFOK model was given by (Goñi et al., 1997), by using the equation ,Eq. (7)

$$C_t = C_\infty \cdot(1-e^{-k\cdot t}) \qquad (7)$$

Where, where C_t (%) is the percent of digested starch at time t (min), C_∞ (%) is the total digestible starch (*i.e.* % of starch digested at ∞ time), and k (min^{-1}) is the digestion rate constant. The starch digestion can be calculated through plotting natural log of slopes within opposite data points as a function of time given by, where t_0 (min) is starting fraction of first starch digestion, at k_1 (min^{-1}) rate and stops when $C_{1\infty}$ (%) i.e the digested starch reached at t_{int} (min). Now digestion of second starch at k_2 (min−1) rate begin and reaches $C_{2\infty}$ (%). By addition, the total digestion of starches is calculated as $C_\infty = C_{1\infty} + C_{2\infty}$ shown in Eq. (8),

$$C(t) =$$

$$C_{1\infty}\cdot(1-e^{-k_1\cdot t}), \text{ if } t\le t_{int} \qquad (8)$$

$$C_{1\infty}\cdot(1-e^{-k_1\cdot t_{int}}) + C_{2\infty}\cdot(1-e^{-k_2\cdot(t-t_{int})}), \text{ if } t \ge t_{int}$$

Parallel kinetics model determines the digestion of starch fractions occurring from t_0 (min) time fraction to extent (%, *i.e.* $C_{1\infty}$, $C_{2\infty}$) with digestion rate k (min−1, i.e. k1, k2). The equation used is Eq. (9)

$$C(t) = C_{1\infty} \cdot (1 - e^{-k_1 \cdot t}) + C_{2\infty} \cdot (1 - e^{-k_2 \cdot t}) \quad (9)$$

Photocatalytic dye degradation

The potential of dye reduction by starch-based adsorbent was evaluated for methylene blue, indigo carmine, and crystal violet by using the method given by(Wawrzkiewicz, Podkościelna, and Tarasiuk 2025) with slight modifications. To make a mixture for dye reduction, starch 0.5g was added to 100mL of water, and then it was mixed with a 10-ppm dye solution. These mixtures containing various dyes were continuously stirred by using a magnetic stirrer under the sun until it reached adsorption and desorption equilibrium. At various time intervals, 2mL aliquots of each were drawn and measured by using a UV-visible spectrophotometer (Shimadzu UV-1800). The removal rates of dyes along with the adsorption capacities were determined using Eq.(10)

$$\%\ \text{degradation} = \left[\frac{Co - Ct}{Co}\right] \times 100 \quad (10)$$

C_o is the initial absorbance of dye at the beginning at 0min., C1 signifies the absorbance measured at a specific time t during the degradation process.

Chapter 6

Result and Discussion

CHAPTER 6

5 Results and Discussion

6.1 Proximate composition of red cowpea flour

According to the methods outlined by AOAC (Howitz & Horwitz, 2019), the proximate analysis of Red cowpea (*Vigna unguiculata)*, resulted in the moisture content at 10.51±0.39% and 0.38±0.03% fat content along with 3.07±0.60% ash and 20.49±0.51% protein, while the sample consists of 65.37±0.63% carbohydrate by difference from fat, ash, protein and moisture content, and crude fiber at 2.04±0.02%. The obtained results align with Hamid et. al. (Hamid et al., 2015). The higher carbohydrate content determines a higher yield rate of starch. These values are summarized in Table 1.

6.2 Starch yield

The starch yield was calculated at 35.12±0.07%, which aligns with similar results given by (Joseph et al., 2024; Padhi et al., 2022). The yield obtained from brown and white cowpea varieties was higher compared to the red variety, at 45.49±0.66% and 46.33±0.00% (Longvah et al., 2017). However, starch yield is higher when compared to some peas,26±0.07% and 30±0.07% for grass and green pea (Ren et al., 2021). Starch yield is influenced by temperature, pH, maturity, and variety of raw materials.

Table 4 - Proximate analysis of red cowpea starch

Results are expressed as means ± standard deviation (n = 3)

RLS- Red Lobia Starch, MRLS- Modified red lobia starch

6.3 Amylose content

Parameter	**(%)**
Carbohydrate	63.73 ± 0.12 %
Amylose Starch (RLS)	33.01 ± 0.15 %
Amylose Starch (MRLS)	27.65 ± 0.27 %
Protein	20.46 ± 0.09 %
Moisture	10.31 ± 0.06 %
Ash	3.05 ± 0.09 %
Fiber	2.17 ± 0.07 %
Fat	0.29 0.06 %

The amylose content was recorded at 33.00±0.14% which is similar to those mentioned in the previous report on the cowpea starch (Huang et al., 2007; Ma et al., 2017). The value of amylose content came out in the range of 30-40% for the Korean variety of cowpea and mungbean starches as stated by Kim et al. (Y.-Y. Kim et al., 2018). However, for mungbean and poroso millet starch from Beijing, China, the values ranged between 28.45-31.80% (Qiao et al., 2024) which is less pure compared to already mentioned. Whereas, for modified starch, the amylose content decreased and calculated at 27.65±0.27% due to hydrolysis of amylose chain and ester formation (Soetan, Falola, and Nwokocha 2017).Amylose content differs due to differences in variety, environmental conditions, and extraction process, and it affects the properties of starch.

7. Application of starch

7.1 Photocatalytic dye degradation

Dye degradation was done by using three dyes, i.e, Methylene blue, crystal violet, and indigo carmine, under 12pH conditions where the fastest dye reduction can be observed under natural sunlight. Dye was reduced by using maleic acid modified as well as native starch within 60min. respectively. Dyes had fast reduction capacity, followed in modified adsorbent material due to increased surface area and changes in the starch structure that created more binding sites for the pollutant. Kinetic models were also applied for determining the degradation by 0th, 1st, and

2^{nd} order kinetics. Among these, the 0^{th} order reaction had the highest R^2 value for MB ($R^2 = 0.9882$), CV fits 1st order ($R^2 = 0.9991$), and IC fits 0th order ($R^2 = 0.9926$).

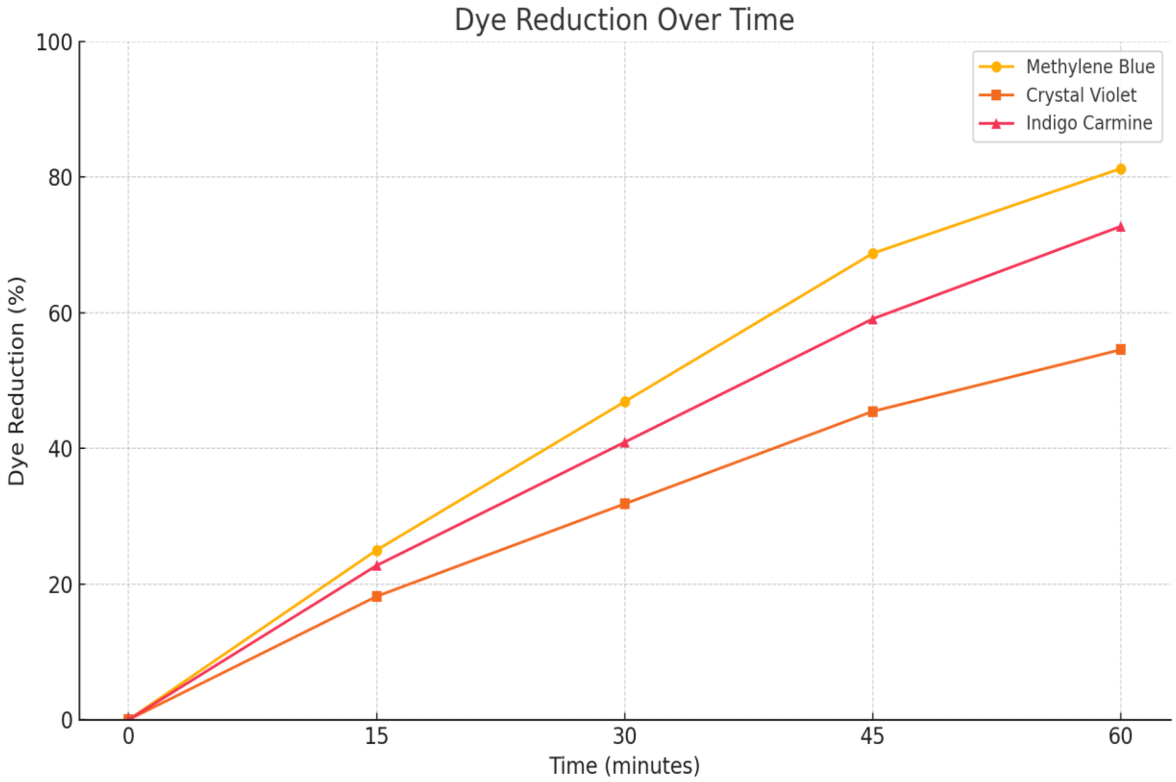


Fig. 6- (A) **Dye degradation** pattern of MB under photocatalytic degradation conditions modified starch ,(B) Dye degradation pattern in native starch (C) Dye degradation pattern of CV under photocatalytic degradation conditions modified starch (D) Dye degradation pattern of IC under photocatalytic degradation conditions modified starch.

Time	**Methylene Blue**	**Crystal Violet**	**Indigo Carmine**
0 min	0.00%	0.00%	0.00%
15 min	25.00%	18.18%	22.73%

30 min	46.88%	31.82%	40.91%
45 min	68.75%	45.45%	59.09%
60 min	81.25%	54.55%	72.73%

Table 5- % degradation calculation for MB, CV and IC

Sample	**Expression**	**Kinetics**	**R^2**
MB	$C_t=C_0-kt$	0th	0.98
	$\ln\left[\frac{Co-Ct}{Co}\right]= -kt$	1st	0.98
	$1/C_t=1/C_0+kt$	2nd	0.88
CV	$C_t=C_0-kt$	0th	0.98
	$\ln\left[\frac{Co-Ct}{Co}\right]= -kt$	1st	0.99
	$1/C_t=1/C_0+kt$	2nd	0.98
IC	$C_t=C_0-kt$	0th	0.99
	$\ln\left[\frac{Co-Ct}{Co}\right]= -kt$	1st	0.98
	$1/C_t=1/C_0+kt$	2nd	0.92

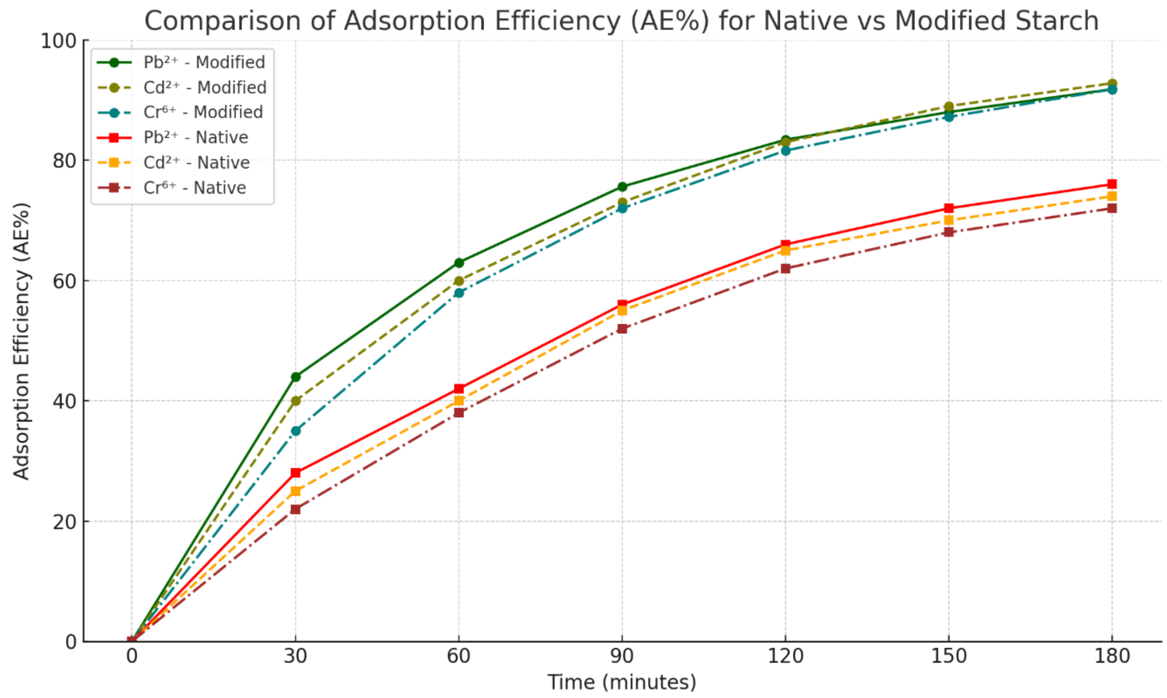
Table 6- Kinetic model of dye reduction

7.2 Heavy metal Adsorption and Kinetic model

Time (min)	**Pb^{2+} AE% (Modified)**	**Cd^{2+} AE% (Modified)**	**Cr^{6+} AE% (Modified)**

0	0.0%	0.0%	0.0%
30	44.0%	40.0%	35.0%
60	63.0%	60.0%	58.0%
90	75.6%	73.0%	72.0%
120	83.4%	83.0%	81.6%
150	88.0%	89.0%	87.2%
180	91.8%	92.8%	91.8%

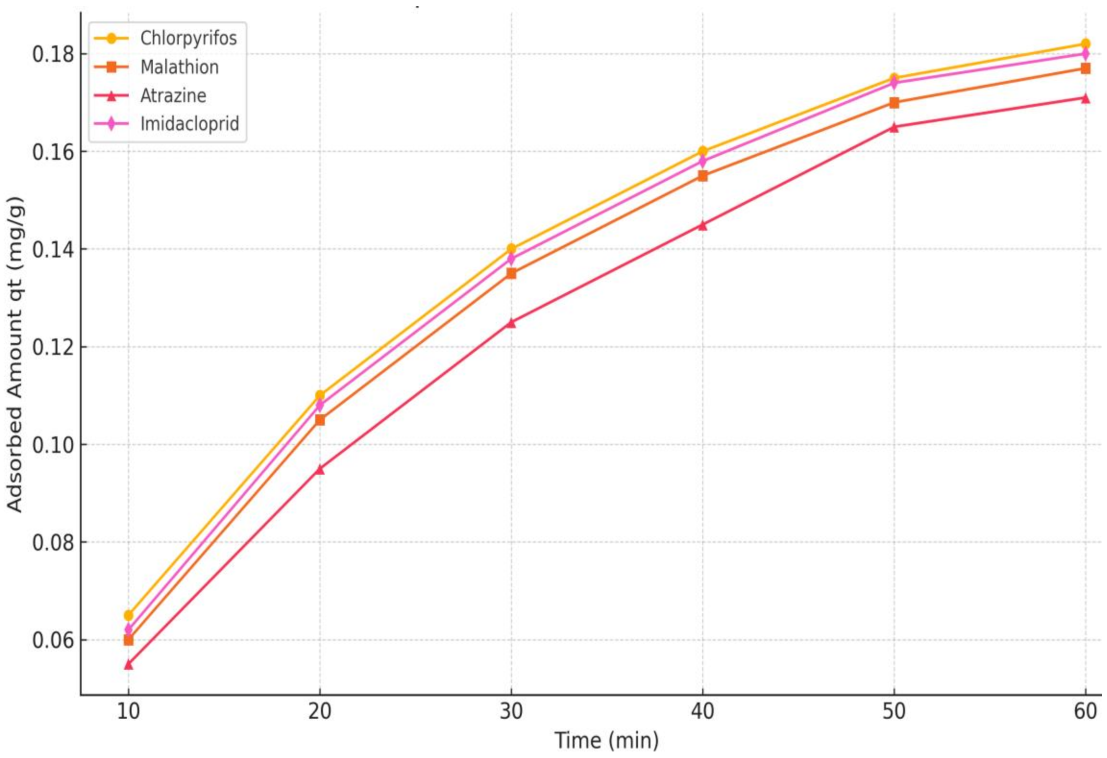
Metal Ion	**Zero Order**	**First Order**	**Second Order**
Pb^{2+}	0.828	0.744	0.744
Cd^{2+}	0.867	0.695	0.695
Cr^{6+}	0.882	0.655	0.655



7.3 Pesticide Adsorption and Kinetic model

Pesticide	**Initial Conc. (ppm)/Dose (50g/L)**	**pH**	**Contact Time (min)**	**Removal Efficiency (%)**
Chlorpyrifos	10	6.5	60	91.2%
Malathion	10	7.0	60	88.5%
Atrazine	10	6.0	60	85.7%
Imidacloprid	10	7.0	60	90.1%

Pesticide	**R^2 (First Order)**	**R^2 (Second Order)**
Chlorpyrifos	0.931	0.997
Malathion	0.912	0.994
Atrazine	0.894	0.991
Imidacloprid	0.921	0.996


Chlorpyrifos
Malathion
Atrazine
Imidacloprid
Adsorbed Amount qt (mg/g)
0.18
0.16
0.14
0.12
0.10
0.08
0.06
10
20
30
40
50
60
Time (min)

Chapter 7

Summary

7 Conclusion and future perspective

The analysis of maleic acid-modified starch revealed specific functional and structural differences and enhancements. The modification introduced carboxyl functional groups through esterification, improving lipid binding and hydration properties. Various characterization techniques showed evident changes due to hydrogen bonding patterns within the structure, and the amorphous region also increases. The enhanced colloidal stability and the surface charge were revealed by zeta potential supporting the application which requires long-term stability and dispersion. This process maintained the structure of the glycosidic backbone of starch along with functional improvement. It resulted in raw materials being used in various sectors such as stabilizers, thickeners, and controlled release agents and it provides overall health and wellness by improving the antioxidant and anti-inflammatory activities and exhibiting shear and thermal stability, making it suitable for food processing applications. The starch can also be used as a dietary formulation as it reduces the enzymatic digestibility, which slowly releases glucose highly recommended for sustained energy release. In addition, future studies can focus on nanomaterials, bioplastics, and composite formation of natural fibers for developing high-performance biodegradable packaging materials, also isothermal and kinetic modeling can be included for enhancing the eco-friendly potential of adsorbents for heavy metals, dyes, and other pollutants in water. Incorporating the modified starches into the pH-responsive delivery system, smart hydrogels or wound dressing can be used in biomedical science applications. Lastly, the advancements and continued functionalization of starch-based materials have an immense future for developing innovation in sustainable technologies, bridging the gap between native biopolymers and advanced new-age applications in various industries.

Chapter 8.

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9. References

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