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Synthesis and physicochemical characterization of derivatized xylan-based pH-sensitive green adsorbent hydrogels for potential sustainable and targeted delivery of drugs

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Abstract

Derivatives of xylans, namely carboxymethyl xylans (CMX), have garnered scientific interest for their potential application as raw materials in the synthesis of biodegradable pH-sensitive green adsorbent hydrogels. The hydrogels were successfully synthesized, as verified by FTIR spectroscopy. The hydrogel with CMX to AA and a weight-by-weight ratio of 1:5 exhibited a maximum swelling percentage of 1534 ± 15.5% in distilled water, maximum porosity (94.26 ± 4.32%), exceptional mechanical strength, and favorable in vitro degradation patterns (12.39 ± 2.46%), which are useful in identifying optimal hydrogels for sustained and targeted drug delivery. The gel-mass fractions were measured to ascertain the physical properties of the gel. Pore diameters were morphologically examined using a scanning electron microscope. This synthesized hydrogel is expected to serve as a powerful substrate for drug delivery and may possess considerable potential in materials science and therapeutic applications.

1.INTRODUCTION

Hydrogels are 3D networks of polymers that are linked together either physically or chemically and can hold water without dissolving it (Bercea et al., 2022). The interpenetrating network often mimics the extracellular matrix, and therefore it is widely used in sustained and targeted drug delivery, tissue engineering, wound healing, biomedicine, targeted release of vitamin B12, and other biological applications (Kundu and Banerjee, 2019; Forero-Doria et al., 2020; Zhang et al., 2023). The hydrogels were prepared by various researchers using a limited supply of petroleum-based raw materials, such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP), and exhibit poor biodegradability and biocompatibility. Therefore, to improve these properties, the utilization of bio-based polymers in hydrogel preparation is attracting considerable attention. In the present scenario, it is essential to develop biopolymer-based hydrogels for various applications. Among these biopolymer-based hydrogels, polysaccharide-based hydrogels are widely used for their biological functions, biodegradability, and low cost (Gao et al., 2016; Hu et al., 2023).

The most common plant-based polysaccharides, like xylan-rich hemicellulose, are made up of 80 to 200 D-xylopyranosyl units joined together by ß (1-4) glycosidic bonds to which O-acetyl and glucuronic acid are attached as side groups. The side groups attached to the xylan backbone are more susceptible and removed during alkaline treatment. These naturally occurring xylans have poor water solubility, antibacterial activity, and mechanical strength that limit their further applications. Therefore, these properties can be enhanced by derivatizing the bio-based polymers like xylan. The D-xylopyranosyl units contain two free hydroxyl groups that can be modified for various applications, particularly in hydrogel-based drug delivery systems. It can thus be derivatized by chemical reactions such as etherification, cross-linking, esterification, and oxidation to improve its suitability (Peng et al., 2012) for the preparation of pH-sensitive hydrogels or other biomedical

applications. Among these chemical modifications, etherification, such as carboxymethylation of xylan, is the most versatile modification for producing biobased materials such as hydrogels that can be used in the paper, textile, paint, and pharmaceutical industries (Heinze & Koschella, 2005; Methacanon et al., 2003; Hu et al., 2023). This fact is because carboxymethylation excludes the use of extreme modification conditions and solvents, which makes it industrially attractive (Konduri and Fatehi, 2016). The sodium salt of carboxymethyl xylan (CMX) can dissolve in water, making it useful as a thickener, helping to mix ingredients, stabilizing mixtures, and keeping particles suspended in products like paints, detergents, and medicines. The carboxymethylation reaction is a process similar to Williamson etherification. It happens when an alkali is present and involves replacing a hydroxyl group in xylan with a part of an alkyl halide called monochloroacetic acid. Adding alcohol (like methanol, ethanol, or isopropanol) to an alkaline water solution helps increase both the amount of substitution and the evenness of functional groups in xylan. Thus, the reaction yield depends upon the reaction temperature, reagent amount, alkali amount, and sequence of process steps. The yield improves when more reagent is added, and the temperature is set to encourage the reaction between the reagent and xylan rather than the reagent breaking down into sodium glycolate or disodium diglycolate (Bogner et al., 2024).

The aim of the present work was to extract xylan (Kumar et al., 2010), followed by carboxymethylation to synthesize CMX with the most suitable parameters, as mentioned by Petzold et al. (2006). CMX was taken as a substrate in varying ratios with acrylic acid for the synthesis of pH-sensitive hydrogels by keeping other parameters like MBA (cross-linker), initiators (potassium persulfate and sodium sulfite), solvent, and temperature constant. The novelty of this work is to synthesize hydrogels using CMX as a substrate and potassium persulfate as an initiator instead of ammonium persulfate.

The current research aligns with the United Nations Sustainable Development Goal (SDG)-3, which emphasizes "Good Health and Well-being." Moreover, it is consistent with the recently ratified BioE3 policy (Biotechnology for Economy, Environment, and Employment), which endorses the biomanufacturing of precision therapies customized to address the specific requirements of individual patients.

2. MATERIAL AND METHODS

Sodium monochloroacetate (SMCA) (AR, 97.5%), N, N′-methylenebis(acrylamide) (MBA) (AR, 99%), potassium persulfate ($K_2S_2O_8$) (AR, 98%) and sodium sulfite (Na_2SO_3) (AR, 95%), acrylic acid (AR, 98%), sodium hydroxide (NaOH) (97%), 2-propanol (AR, 99%), absolute ethyl alcohol (C_2H_5OH) (AR, 99.5%), and buffer tablets (pH 7) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. The other chemicals were of analytical grade, and distilled water was used throughout the experiment.

2.1 Raw Material

The maize cobs were collected from a nearby agricultural area in Satna, Madhya Pradesh, India. The maize cobs were ground into the powder, and the fractions that were retained on 80 mesh (177 µm) screens after passing through 40 mesh (400 µm) screens were collected.

2.2 Isolation of xylan

The powdered corn cob was utilized to extract xylan following the methodology of Kumar and Negi (2014) and Kumar et al. (2017) with some modifications. The powdered corn cob was rendered extractive-free utilizing a mixture of organic solvents (ethanol:toluene::2:1) in accordance with TAPPI standard T264 om-07, thereafter undergoing delignification with acidified NaClO2, classified as holocellulose. The holocellulose was subsequently treated with a 2.5N NaOH solution to dissolve a specific component at ambient temperature. The dissolved component in the filtrate was precipitated through neutralization with acetic acid and subsequently settled using ethanol. The obtained product was filtered and thoroughly washed with ethanol, then dried at 60°C until a consistent weight was achieved. The resultant product was identified as xylan and preserved for modification or derivatization.

2.3 Synthesis of carboxymethyl xylan (CMX) through homogeneous activation of xylan

The extracted maize cob xylan was carboxymethylated using the modified procedure outlined by de Mattos et al. (2019). This research selected conditions for carboxymethylation to achieve highest yield percentage, conducted in a homogeneous reaction medium. The synthesis of CMX using a homogenous activation procedure was conducted as follows: 2 g (15.26 mmol) of xylan was dissolved in 10 mL of a 25% (w/v) NaOH solution (2.4 g; 60 mmol), then followed by the addition of 14 mL of 2-propanol. The mixture was agitated for 3 hours at ambient temperature, after which 7 g (60 mmol) of sodium monochloroacetate (SCMA) was introduced, and the temperature was elevated to 75°C for 70 minutes. The resultant mixture was neutralized with 65% (v/v) acetic acid, subsequently followed by precipitation using ethanol. The resultant precipitate was rinsed thrice with 50 mL of 65% ethanol (v/v) and subsequently dried in a vacuum oven until a consistent weight was achieved.

The CMX yield was determined based on the dry weight of xylan, as outlined in Equation (1).

$$\text{Yield (\%)} = \frac{\text{Experimental mass [MExp(CMX)]}}{\text{Theoretical mass [MTheo(CMX)]}} \times 100 \quad \text{Eq (1)}$$

Where[MExp(CMX)] is the experimental mass of dried CMX (g) and [MTheo(CMX)] is the theoretical mass of of CMX (g) on oven dried basis. The percentage yield of CMX synthesis was found to be 83.40±1.60 %.

2.4 Synthesis of carboxymethyl xylan (CMX) through heterogeneous activation of xylan

The carboxymethylation of xylan was conducted according to the methodology outlined by de Mattos et al. (2019), focusing on maximizing yield % using a heterogeneous reaction medium with slight modifications as detailed below: The synthesis of CMX by heterogeneous activation was conducted using 2 g (15.26 mmol) of xylan suspended in 60 mL of 2-propanol for thirty minutes under stirring. Subsequently, 40 mL of a 15% NaOH solution (w/v) (6 g, 150 mmol) was introduced with vigorous agitation at ambient temperature for 1 hour. Subsequently, 7 g (60 mmol) of sodium monochloroacetate (SMCA) was introduced at 55°C for a duration of 6 hours. The result was filtered, suspended in 80% methanol (v/v), neutralized with weak acetic acid, and washed six times with 20 mL of ethanol. Subsequent to this treatment, the precipitate was subjected to drying in a vacuum oven until a consistent weight was achieved for further characterization. The percentage yield of CMX was determined using equation 1, yielding a value of 54.9 ± 3.77 %.

2.5 Degree of Substitution

The degree of substitution is defined as the average number of hydroxyl groups replaced in each D-xylose unit of the polymeric chain. In an isolated biomolecule, such as xylan, each xylopyranose unit contains two available hydroxyl groups. The maximum degree of substitutions is two, as only two acetyl groups can be connected to a xylopyranose unit.

The substitution degree of CMX was ascertained using the complexometric titration method outlined by de Mattos (2019). 0.5 g of oven-dried CMX was placed in a conical flask, followed by the addition of 50 mL of distilled water and 12.5 mL of 0.3 M NaOH solution. The solution mixture was heated to boiling for 15 minutes. The entire reaction mixture was titrated with a 0.3 M HCl standard solution using phenolphthalein as an indicator.

The substitution degree was determined according to equation (2).

$$A = (BC - DE)/F \quad \text{.....Eq (2)}$$

Where, 'A' is the milliequivalents of acid consumed per g of sample; 'B' is the Volume of NaOH solution added (mL); 'C' is the Molarity of NaOH solution; 'D' is the Volume of HCl solution required for titration of the excess NaOH solution (mL)

$$DS = \frac{0.132 \times A}{1 - 0.058 \times A} \quad \text{... ..... Eq (3)}$$

Where, DS is the Degree of substitution and 0.132 is the molecular mass of one unit of anhydroxylose; 0.058 is the net increase in the molecular mass of the anhydroxylose unit for each carboxymethyl group added after carboxymethylation

2.6 Synthesis of carboxymethyl xylan (CMX)-based hydrogels

CMX (0.5 g) was placed in a 50 mL beaker, to which 10 mL of distilled water was added. The mixture was then heated to 60 °C in a water bath with continuous stirring to ensure complete dissolution. Subsequent to the dissolution of the CMX, 0.1 g of each redox initiator, $K_2S_2O_8$ and Na_2SO_3, was added, and the temperature of the mixture was sustained at 40 °C for a minimum duration of 10 minutes. Afterwards, differing quantities (0.5 g to 4 g) of acrylic acid were included, and the temperature was elevated to 50 °C. Subsequently, 0.05 g of N,N-methylenebisacrylamide (MBA) was incorporated with continuous agitation for 30 minutes, after which the mixture was maintained in a water bath at 50 °C. The gel product was obtained and thoroughly cleansed by immersing it in distilled water, with the water being replaced every 6 hours for a duration of 24 hours. Subsequent to thorough washing, the gel products were sectioned into cubic fragments and subsequently dried in a vacuum oven for further analysis.

2.7 Physicochemical characterization of synthesized derivatized xylan-based hydrogels

The physicochemical characterization of the derivatized xylan-based hydrogels is essential to confirm their successful synthesis, understand structure–property relationships, and evaluate suitability for applications, specifically in the delivery of drugs to the intended location.

2.8 Polymer yield

The grafting parameters of the CMX-based hydrogels were established by immersing the dried weight of the hydrogel sample in distilled water at room temperature for 48 hours to facilitate swelling. The swelled hydrogel samples were thoroughly cleaned to eliminate contaminants, particularly acrylic acid, and subsequently dried at 60°C in a vacuum oven until a consistent weight was achieved. The samples' weight variation was observed, and the grafting parameters, including gel mass fraction (GMF), graft yield (GY), and graft efficiency (GE), were calculated according to Eqs. (4), (5), and (6), respectively.

$$\% \text{ GMF} = \frac{Wg}{Wd} X\ 100 \quad \text{.....Eq (4)}$$

$$\% \text{ Graft yield} = \frac{Wg - Wo}{Wo} X\ 100 \quad \text{..... Eq (5)}$$

$$\% \text{ Graft efficiency} = \frac{Wg - Wo}{Wm} X\ 100 \quad \text{..... Eq (6)}$$

Where, W_g was the weight of grafted polymer after purification, $W_d$was the weight of synthesized dried grafted polymer, W_0 was the initial weight of the xylan and W_m was the weight of monomer added to the reaction.

2.9 Degree of swelling

The degree of swelling of hydrogel was ascertained using a gravimetric method at room temperature. For the measurement of the degree of swelling, the synthesized CMX-based hydrogels were first cut into small pieces, followed by drying in a hot air oven. The oven-dried cuboid cuts of hydrogels were weighed. The tea bags of 100 mesh nylon screen containing accurately dried weight hydrogel samples were immersed in 100 mL of distilled water to swell. Thereafter, swollen hydrogels were removed after 48 hours and weighed. Every experiment was conducted in triplicate, and Eq. (7) was used to calculate average swelling percentage.

$$\text{Swelling } \% = \frac{W2 - W1}{W1} \text{ x } 100 \text{.....Eq (7);}$$

Where; W_1 and W_2 are the dried weight and swollen weight of hydrogels, respectively.

2.10 Porosity

The liquid displacement method helps to determine the open porosity of hydrogels (Karageorgiou and Kaplan 2005; Sharma et al. 2021). A series of rapid evacuation-repressurization cycles were employed to force the water into the hydrogel's pores, which was placed in a known volume (V1) of distilled water. The total volume of impregnated hydrogels in distilled water was designated as V2. The volume that remained after the removal of impregnated hydrogels was considered V3. Thus, the volume of voids where water was absorbed resulted as V1-V3, and the bulk volume of hydrogels was V2-V3. Thus, porosity was defined as the ratio of (V1 – V3) to (V2 – V3), and it was calculated using Eq. (8) as shown below:

$$P = \frac{V1 - V3}{V2 - V3} x\ 100 \quad \text{.....} \quad \text{Eq (8)}$$

2.11 *In vitro* degradation

The degradation patterns of different ratios of manufactured CMX-based hydrogels were analyzed by monitoring alterations in wet weight over time. To determine the degradation trends, the dried hydrogels were weighed, and each sample was incubated in a shaking incubator at 150 rpm and 37 °C in a tissue culture plate containing 5 mL of PBS (pH 6.8). Every five days, hydrogels were removed from the wells and weighed after the surface water was absorbed with filter paper to monitor weight variations over a period of 65 days (Kayabolen et al. 2017; Sharma et al. 2021). Additionally, the PBS was substituted every

fifth day, and the weight loss of each hydrogel was calculated using Equation (9).

$$\text{Weight loss} = \frac{wi-wf}{\text{wi}} x\ 100 \quad \text{.....Eq (9)}$$

Where wi represents the starting weight of hydrogels in their swelled state in PBS, and wf reflects the weight of the deteriorated hydrogels at each five-day interval.

2.12 Instrumental analysis for characterization of synthesized CMX-based hydrogels

Instrumental analysis is decisive to determine their chemical structure, mechanical strength, thermal stability, morphology and porosity.

2.13 Study of interaction pattern of functional groups

An FTIR analysis was conducted on xylan, CMX, and CMX-based hydrogels to identify the functional groups contained in the materials utilizing an FTIR spectrophotometer (Model: FTIR-8900, Shimadzu, Japan). The investigation was conducted within the wavenumber range of 4000 cm^{-1} to 400 cm^{-1} against % transmittance by fabricating KBr pellets.

2.14 Analysis of mechanical strength

The mechanical strength of the synthesized CMX-based hydrogels was assessed by compression stress and modulus measurements. The Instron Universal Testing Machine (UTM)-3315, an electromechanical material testing apparatus, was utilized to assess characteristics such as compression stress and modulus of the hydrogels. The examination was performed at ambient temperature (25 °C), 60% relative humidity, and a crosshead speed of 2 mm/min. The specimen, measured at 10 mm x 12.7 mm x 12.7 mm, was preloaded with a 1 N force to mitigate the effects of surface artifacts. Upon failure, stress and modulus were recorded and calculated based on the original cross-section.

2.15 Study of internal structure

The morphology of the generated CMX-based hydrogels was examined using Field Emission Scanning Electron Microscopy (FE-SEM: Mira3 Tescan model) at an acceleration voltage of 10 kV. The entrapped water in the expanded hydrogels was eliminated through freeze-drying, followed by cross-sectioning, and then sputter-coated with a gold layer for one minute in an autofine coater unit (JFC-1600) before to being mounted on the FE-SEM. The samples' micrographs were captured at various magnifications, and the picture at 10,000× was analyzed using the ImageJ software (NIH, Bethesda, Maryland, USA) to ascertain the mean and distribution of pore size.

3. RESULTS AND DISCUSSION

3.1 Isolation of xylan

The fractions of powdered maize cobs maintained on 80 mesh (177 µm) screens, following passage through 40 mesh (400 µm) screens, were utilized to extract xylan according to the procedure outlined by Kumar et al. (2022). The percentage yield of xylan was determined on an oven-dry basis of holocellulose, yielding a value of 5 ± 1.2%.

3.2 Synthesis of carboxymethyl xylan (CMX) via homogeneous and heterogeneous activation of xylan

The carboxymethyl xylan was prepared by both homogeneous and heterogeneous activation processes. The average percentage yield of the product was found to be maximum (83.40 ± 1.60%) through the homogeneous activation as compared to heterogeneous (54.9 ± 3.77%) one (Figure 1). In the homogeneous activation, the xylan was first dissolved in an alkaline solution of NaOH, followed by the addition of 2-propanol. The 2-propanol having a low dielectric constant may immobilize the NaOH near the xylan surface prior to the reaction, which could result in a long chain of CMX in the final product. Putting NaOH nearby to the surface of xylan probably increased the number of collisions between NaOH and xylan, which sped up the reaction and increased the yield. As a result, the homogeneous activation strategy (Figure 2) raised the percentage yield of CMX more than the heterogeneous one.

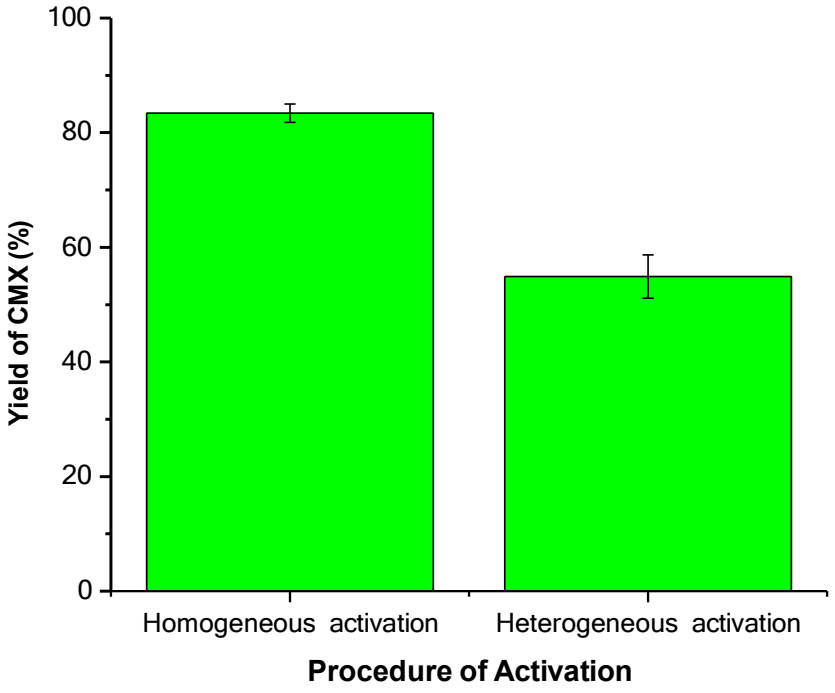


Figure 1. Synthesis of carboxymethyl xylan (CMX) via homogeneous and heterogeneous activation of xylan

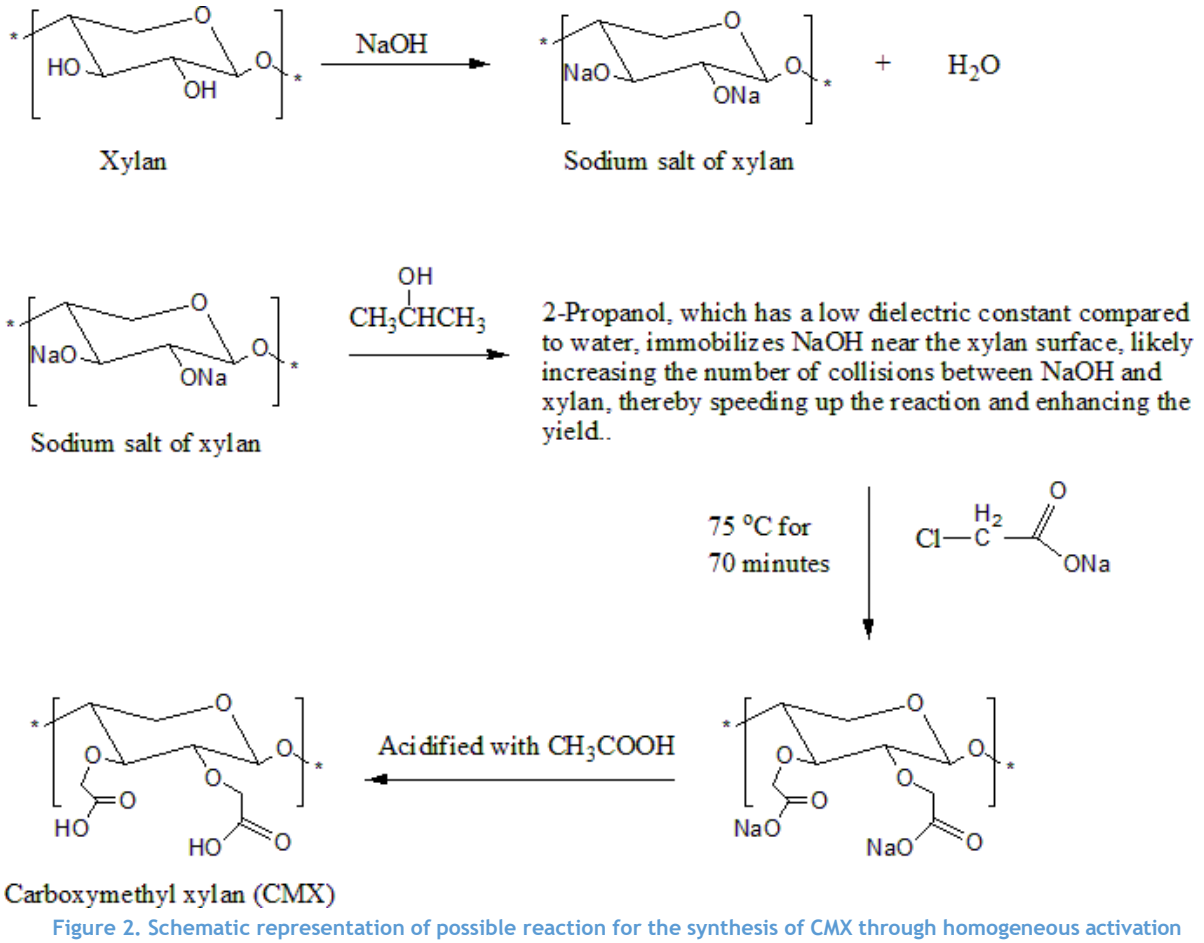


Figure 2. Schematic representation of possible reaction for the synthesis of CMX through homogeneous activation

3.2.1 Degree of Substitution

The degree of substitution (DS) of CMX achieved through homogenous activation was markedly higher (1.39 ± 0.01). This results from the utilization of organic suspension media, such as 2-propanol, which facilitates improved dispersion of aqueous NaOH inside the xylan matrix. Improved dispersion elevates hydroxide ion availability for etherification and mitigates unwanted side reactions that result in the creation of sodium salts of glycolic and diglycolic acids, which are challenging to eliminate during purification. Moreover, the low dielectric constant of 2-propanol may concentrate NaOH at the xylan surface before the reaction, thus enhancing reaction efficiency (Pushpamalar et al., 2006). Thus, employing solvents with low dielectric constants to that of water is beneficial for producing CMX with extended polymer chains. The homogeneous activation approach is more efficient than the heterogeneous method, as it increases xylan reactivity by enhancing the accessibility of hydroxyl groups. The DS is essential in regulating the physicochemical characteristics of CMX, such as solubility, emulsifying capacity, acid resistance, and salt tolerance.

3.3 Synthesis of CMX-based hydrogels

CMX-based hydrogels were synthesized according to the procedure outlined in the Synthesis of CMX-based hydrogels section of the Materials and Methods. Acrylic acid and CMX were utilized in varying weight ratios (w/w) from 1:1 to 10:1 for hydrogel synthesis, as depicted in the figure 3.

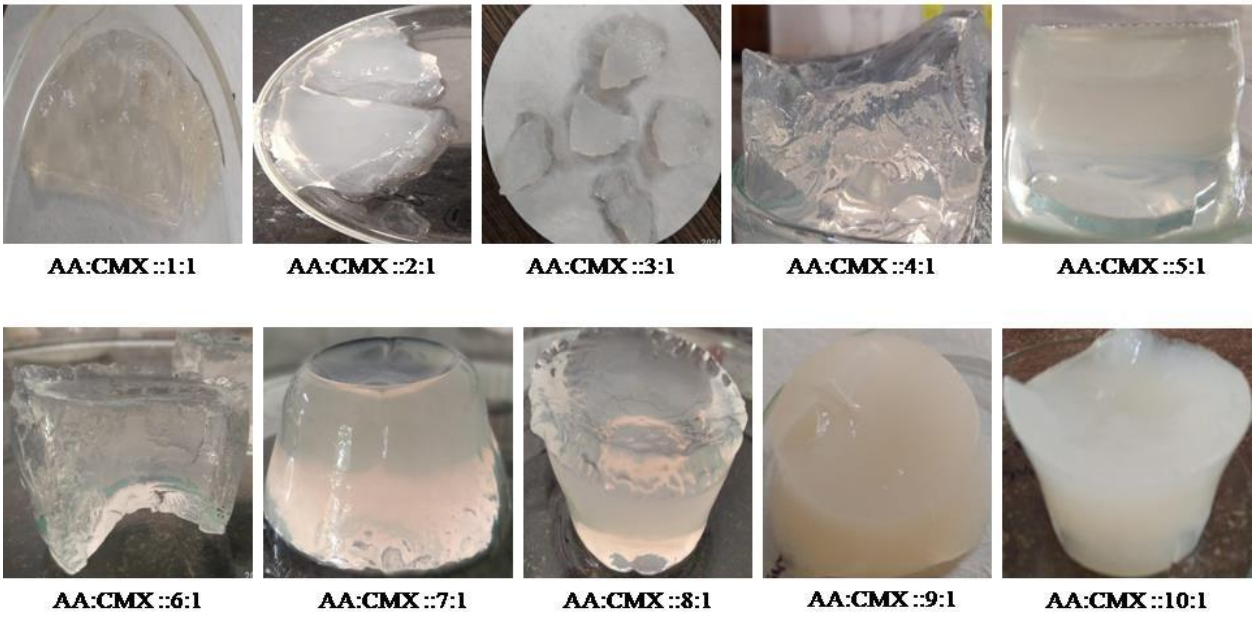


Figure 3. Photographs of the synthesized CMX-based hydrogels of varying weight ratios (w/w) of AA and CMX

The synthesized CMX-based hydrogels were found to be more fragile and transparent than the native xylan-based hydrogels. This behavior may be attributed to the introduction of bulkier substituents, such as carboxymethyl groups, into the xylan backbone. The presence of these carboxymethyl groups likely disrupts the polymeric network and interferes with effective crosslinking, resulting in a more fragile hydrogel structure. Furthermore, the carboxymethylated xylan derivatives exhibited lower swelling capacity compared to the hydrogels prepared from non-derivatized xylan, possibly due to the altered network architecture and reduced crosslinking density.

3.4 Possible reaction mechanisms involved in CMX-based hydrogels

CMX-g-poly(acrylic acid) hydrogels were generated using free-radical graft copolymerization of carboxymethyl xylan (CMX) and acrylic acid (AA) utilizing a crosslinker, N,N′-methylenebisacrylamide (MBA), and a redox initiator system ($K_2S_2O_8/Na_2SO_3$). The proposed reaction mechanism for the formation of the hydrogel network is illustrated in the figure 4. The initiator, potassium persulfate, decomposes in the reaction mixture to generate sulfate anion radicals. These highly reactive radicals can abstract hydrogen atoms from the –OH groups present on the partially carboxymethylated xylopyranose units of the CMX backbone, thereby forming CMX-based free radicals (alkoxy radicals). In addition, the sulfate anion radicals may also react with AA and the crosslinker (MBA), generating secondary free radicals that further participate in the grafting and crosslinking reactions. The secondary (2°) free radicals produced from acrylic acid (AA) monomers subsequently interact with more AA molecules, forming new radical sites on following AA units. The chain-propagation process persists, resulting in the gradual creation of extended poly(acrylic acid) (polyAA) chains. Concurrently, CMX-derived free radicals interact with AA monomers to produce secondary free radicals on the AA units. These radicals are stabilized through resonance and hyperconjugation, facilitating the continued progression of the polymerization cycle. With the incorporation of more AA molecules, fresh secondary radicals are generated on each subsequent monomer unit. This propagation mechanism leads to the elongation of polyAA chains and the synthesis of graft copolymers, specifically CMX-g-poly(acrylic acid) (CMX-g-polyAA). Ultimately, the polymer chains of polyAA, CMX-g-polyAA, and MBA, each possessing 2° free radicals, along with leftover CMX radicals, engage in reciprocal radical coupling. These coupling processes function as termination steps, resulting in the creation of an interconnected three-dimensional polymer network that forms the hydrogel.

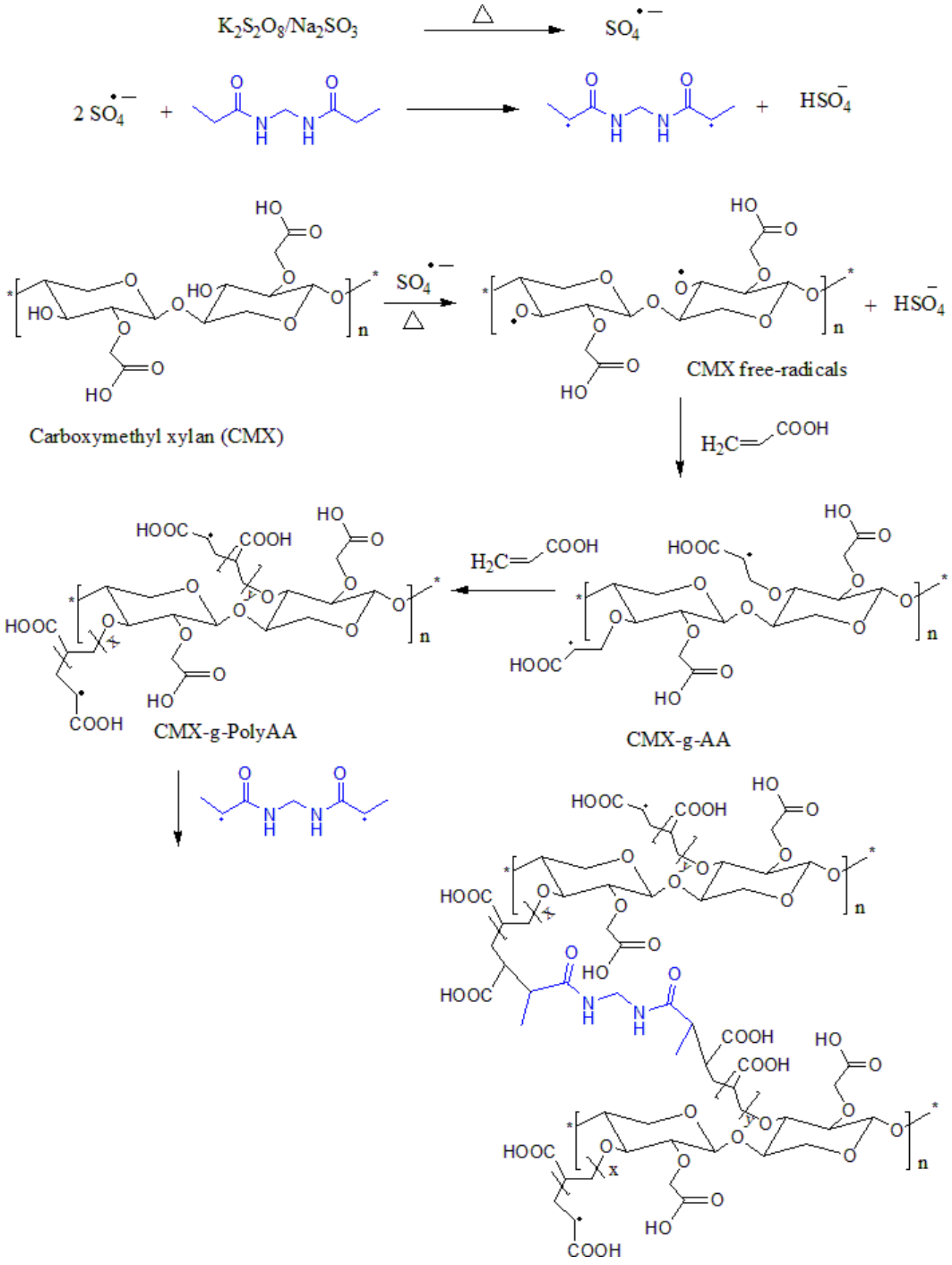

K2S2O8/Na2SO3
SO4
2 SO4
HSO4
Carboxymethyl xylan (CMX)
CMX free-radicals
H2C=COOH
CMX-g-PolyAA
CMX-g-AA

Figure 4. Schematic illustration of the possible reaction mechanisms involved in the synthesis of CMX-based hydrogels via free-radical graft copolymerization.

3.5 Physicochemical Characterization of Synthesized CMX-based Hydrogels

3.5.1 Polymer yield

A relationship between the concentration of acrylic acid (AA) and the gel fraction of the resulting hydrogel was established by determining the gel mass fraction, graft yield, and graft efficiency. These parameters collectively provide valuable insight into the structural integrity, crosslinking density, and overall polymerization efficiency of the hydrogel network.

3.5.2 Gel-mass fraction (GMF) of synthesized hydrogels

The degree of polymer connectivity by physical or chemical cross-linking can be evaluated using the percentage gel mass fraction (%GMF). The findings indicated that %GMF rose with higher acrylic acid (AA) content, attributable to improved cross-linking between CMX and poly(acrylic acid) (polyAA) chains. The hydrogel formed with a CMX to AA weight ratio of 1:5 or greater demonstrated a %GMF of almost 90%, signifying that the graft copolymerization and cross-linking reactions approached completion, resulting in a stable cross-linked hydrogel network. CMX is rich in hydroxyl (-OH) groups, which enhance the formation of extra free-radical sites, thus facilitating graft copolymerization and cross-linking among polymer chains. Increased cross-linking density leads to a more stiff polymer network, which diminishes chain mobility, thereby reducing the available free volume for water absorption and restricting the hydrogel's swelling capacity.

On the other hand % GY was greatly increased and reaches a maximum value (892 ± 3 %) with the highest ratio (w/w) of AA to CMX (Figure 5). This might have happened as the copolymerization reaction was carried out at the same conditions except for the concentration of AA; therefore, it is imperative to assume that the concentration, nature, and efficiency of the free-radicals and other active species generated during the polymerization reaction was the same. The increase in percentage GY on increasing the ratios of acrylic acid within the studied range could be attributed to the greater heaping up of AA molecules in the close proximity of the CMX backbone. The AA molecules in the immediate neighborhood of reaction sites become acceptors for the CMX macro radicals, resulting in chain initiation, and thereafter become free-radical donors to the neighboring AA molecules. As a consequence, the crosslinking density increased, forming a three-dimensional crosslinked insoluble and infusible network and resulting in an increased percentage of graft yield from 110 ± 6 to 892 ± 3, as shown in Figure 5. The grafting efficiency (%) increased with increasing AA concentration, attaining a maximum value of 98.5 ± 5.6 at a 5:1 (w/w) ratio of AA to CMX. Beyond this ratio, the grafting efficiency became nearly constant with a slight decline. This behavior may be attributed to enhanced homopolymerization of AA at higher monomer concentrations. Excess AA promotes increased combination and disproportionation of poly(AA) macroradicals, leading to the formation of homopolymers rather than grafted chains, thereby marginally reducing the grafting efficiency.

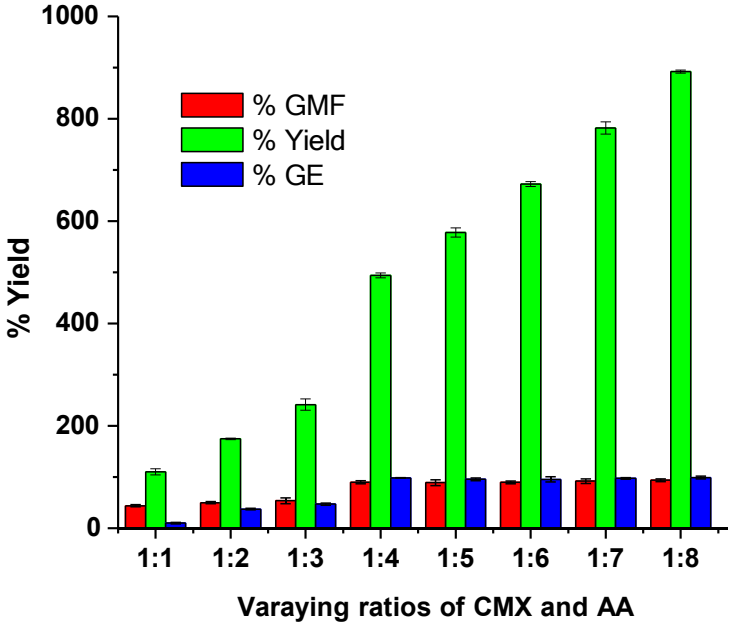


Figure 5. Percentage gel mass fraction (GMF), graft yield (GY), and grafting efficiency (GE) of CMX-based hydrogels prepared at different CMX-to-AA weight ratios.

3.6 Water absorption capacity or Degree of swelling

The degree of swelling in the synthesized hydrogels was assessed by initially cutting the hydrogel samples into small cubic fragments and subsequently drying them in a hot air oven. The oven-dried cuboidal hydrogel samples were subsequently weighed and submerged in 100 mL of distilled water for 48 hours for complete swelling. Following the swelling, the hydrogel samples were removed, excess surface water was eliminated with tissue paper, and the samples were subsequently weighed.

Figure 6 illustrates the swelling percentage of hydrogels produced with different weight-to-weight ratios of CMX to AA. Figure revealed that the degree of swelling was increased with the increasing of AA first and reached a maximum degree of swelling upto 1534 ± 15.5 % with weight ratio of CMX to AA of 1:5 and then after decreased. The swelling percentage was first increased indicating the progress of the reaction towards completion via free radicals generation. Reaching the swelling percentage upto the maximum, homopolymerization will started

and the polymeric chains may become more entangled leads to less available space resulting in a reduction in the accessible volume for swelling to occur. There is a possibility that this approach will cause the swelling percentage to decrease as the concentration of AA increases.

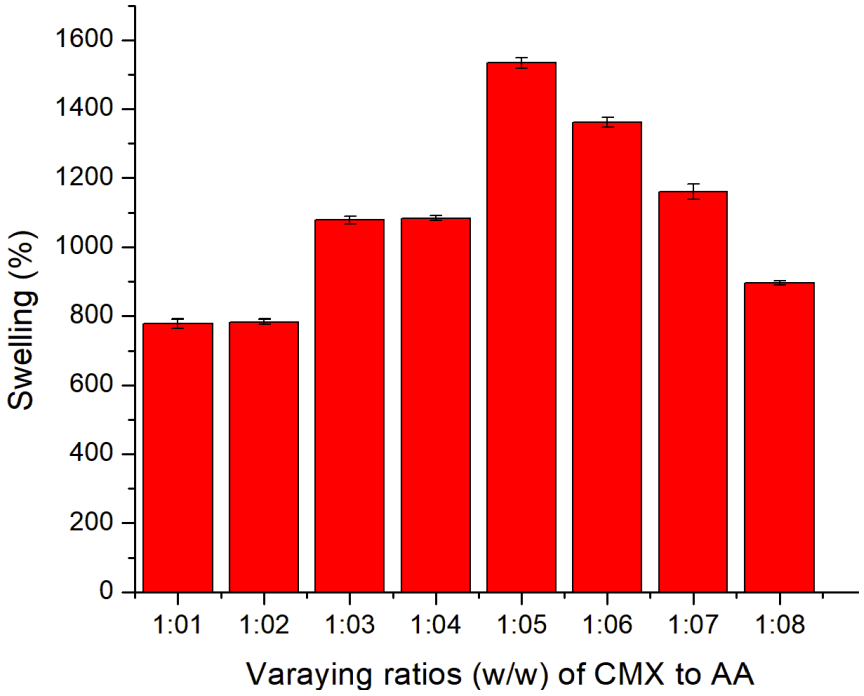


Figure 6. Percentage of swelling of synthesized hydrogels with varying weight ratios of CMX to AA

3.7 Porosity

Porosity is defined as the ratio of the void volume to the bulk volume of the hydrogel. All synthesized hydrogels exhibited high open porosity, approaching approximately 90%. The hydrogel made with a CMX-to-AA ratio of 1:5 (w/w) had a slightly higher porosity of 94.26 ± 4.32%, as shown in Figure 7. This behavior can be attributed to copolymerization proceeding via a free-radical mechanism, resulting in the formation of larger pore structures. However, further increases in acrylic acid content led to the generation of a greater number of free radicals, which promoted increased chain entanglement and crosslinking density. Consequently, a highly compact network with a larger number of smaller-diameter pores was formed, reducing the available void volume and overall porosity. As a result, increasing AA content produced a denser hydrogel matrix in which water molecules are physically entrapped within a tortuous three-dimensional polymeric network.

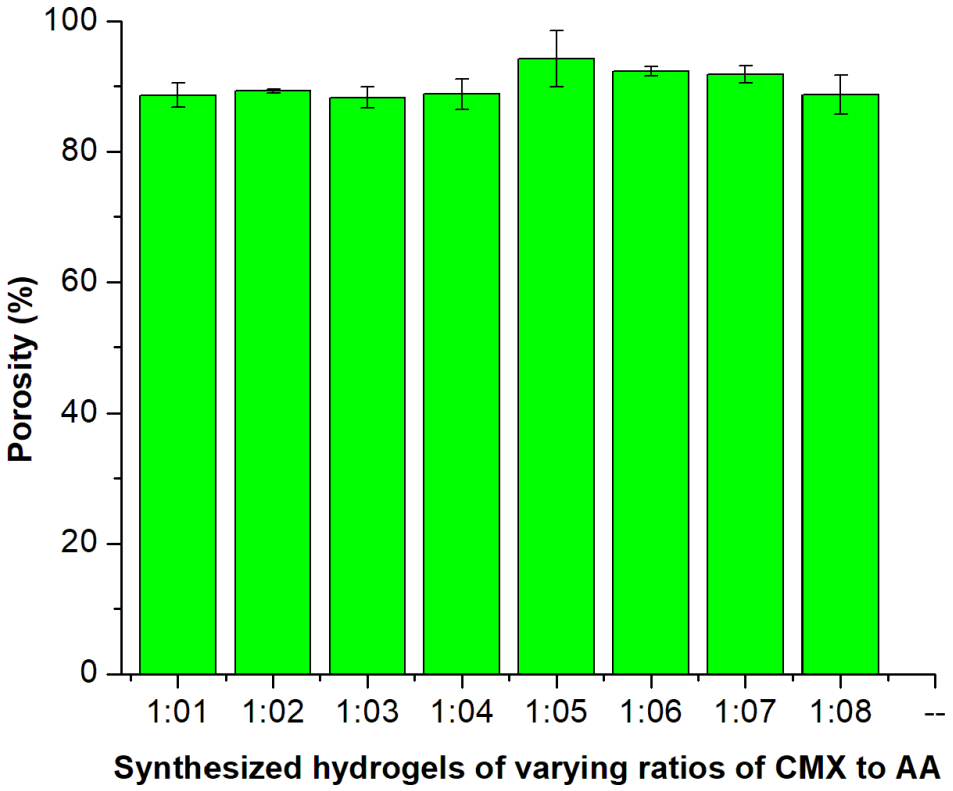


Figure 7. Percentage of porosity of synthesized hydrogels of varying ratios of CMX to AA

3.8 *In vitro* degradation

The *in vitro* breakdown of hydrogels is a critical factor for their use as biomaterials, as it influences essential in vivo properties such as structural integrity in a biological environment and the efficacy of controlled release of bioactive substances. This work assessed the breakdown behavior of hydrogels under meticulously regulated settings utilizing a simulated buffer solution (pH 6.8) at 37 °C. Weight loss was evaluated over 65 days to examine the breakdown of hybrid hydrogels in relation to their acrylic acid (AA) content shown in Figure 8.

The hydrogel with the lowest AA content demonstrated the greatest deterioration, achieving a weight loss of 24.03 ± 2.21 % after 65 days. The degradation profiles significantly differed based on hydrogel composition, demonstrating that CMX presence substantially influences degradation behavior. This observation aligns with prior reports indicating that the amorphous areas of polymeric scaffolds breakdown more swiftly than their crystalline counterparts during hydrolytic degradation (Tan et al., 2009). The greatest weight loss occurred in the hydrogel with a 1:1 (w/w) ratio of CMX to AA, while the most significant swelling behavior was noted in the hydrogel with a 1:5 weight-by-weight ratio of CMX to AA. Increased swelling with enhanced AA content may provide sustained or regulated medication release, thereby enhancing therapeutic efficacy and decreasing dose frequency.

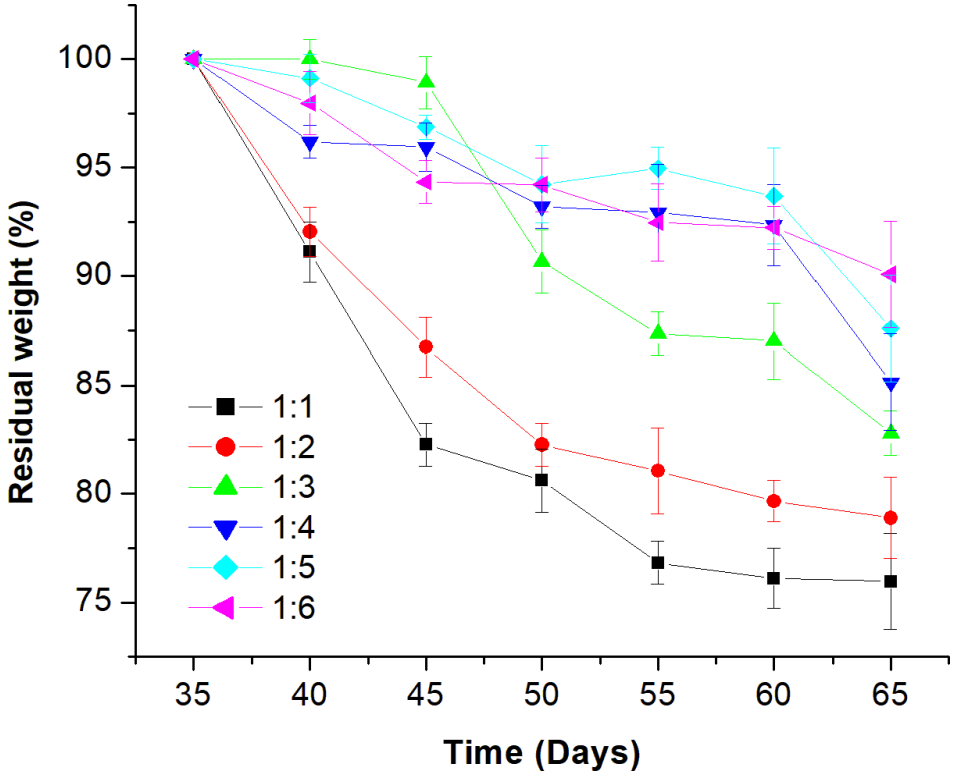


Figure 8. Percentage degradation of varying weight by weight ratio of synthesized hydrogels

3.9 Instrumental Analysis for Characterization of Synthesized Xylan-based Hydrogels Analysis of mechanical strength

Hydrogels intended for colon-targeted drug administration must possess adequate mechanical strength between hundreds of kilopascals (kPa) and several megapascals (MPa), as well as a modulus ranging from 1 to 200 kPa, to endure the physical stresses of the gastrointestinal (GI) tract. The mechanical strength of hydrogels synthesized using CMX and acrylic acid in w/w ratios of 1:4, 1:5, and 1:6 was evaluated using the Instron Universal Testing Machine (UTM), with the findings illustrated in Figure 9. It was noted that when the acrylic acid content increased, both the compressive stress (7.13 ± 0.87 MPa to 3.98 ± 0.73 MPa) and the modulus (54.39 ± 1.23 MPa to 35.82 ± 0.82 MPa) diminished. The results indicate that carboxymethylated xylan (CMX) possesses carboxymethyl groups on its xylan backbone, which impede crosslinking, resulting in a weakened structure that exhibits inferior compression strength under pressure. Consequently, the CMX-based hydrogels with high AA content exhibited increased brittleness. Hydrogels composed of reduced AA content exhibit enhanced water absorption while maintaining structural integrity, rendering them promising subjects for additional investigation. The hydrogel formulated with a 1:5 w/w ratio of CMX to AA was deemed the optimal choice for future drug delivery systems, since it necessitates lower compressive strength to facilitate effective drug release and cellular contact.

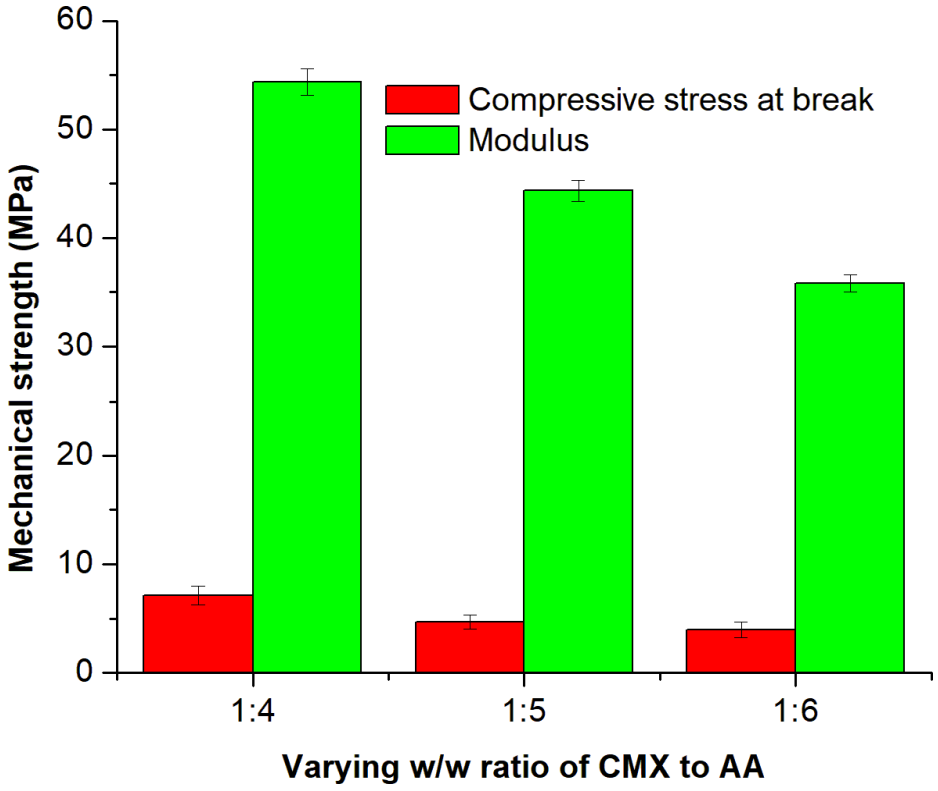


Figure 9. Mechanical strength of synthesized hydrogels with varying molar ratios of CMX to AA.

3.10 Study of the interaction pattern of functional groups using FTIR spectroscopy

Xylan, carboxymethyl xylan (CMX), and the CMX-based synthesized hydrogel were characterized for the presence of functional groups using FTIR in the wavenumber range of 4000–550 cm^{-1}, and results are depicted in Figure 10. The majority of the absorption bands obtained for xylan were similar to those reported by Kumar et al. (2010) and Kumar and Negi (2012a, 2012b, and 2014). In the case of xylan, a broad absorption band was observed in the wavenumber range of 3405 and 3268 cm^{-1}, indicating the stretching vibration of -OH groups caused by the presence of water molecules due to xylan's ability to absorb moisture (da Silva et al. 2012; de Mattos et al. 2019). The absorption bands at 2929 cm^{-1} and 2852 cm^{-1} were associated with asymmetric and symmetric vibrations of -CH_2 groups (Magaton et al. 2008; da Silva et al. 2012).

The primary spectral features were detected within the range of 800 to 1750 cm^{-1}. The band observed at 1641 cm^{-1} was referred to as water absorption vibrations (de Mattos et al. 2019). A sharp absorption band present at 1554 cm^{-1} might be attributed to the C=C stretch of the aromatic ring, confirming the traces of lignin that were likely remnants of lignin carbohydrate complexes (Fonseca Silva et al. 2012). A strong signal at 1404 cm^{-1} corresponds to the bending of methylene groups attached to xylan (Díaz et al. 2024). The specific absorption band at 1037 cm^{-1} was linked to the stretching of C-O and C-C bonds and the ß-glycosidic linkage (C-O-C), which was typical for xylan (Peng et al. 2011; de Mattos et al. 2019). A sharp absorption band at 896 cm^{-1} was observed, which was indicative of the ß-configuration of the 1→4 glycosidic bond between xylopyranose units of the main xylan chains (Buslov et al. 2009). A sharp absorption band at 1595 cm^{-1} observed in carboxymethyl xylan indicates the asymmetric stretching vibration of the carboxylate group (-COO^-), in addition to the characteristic absorption bands of xylan. This confirms the successful synthesis of carboxymethyl xylan (CMX).

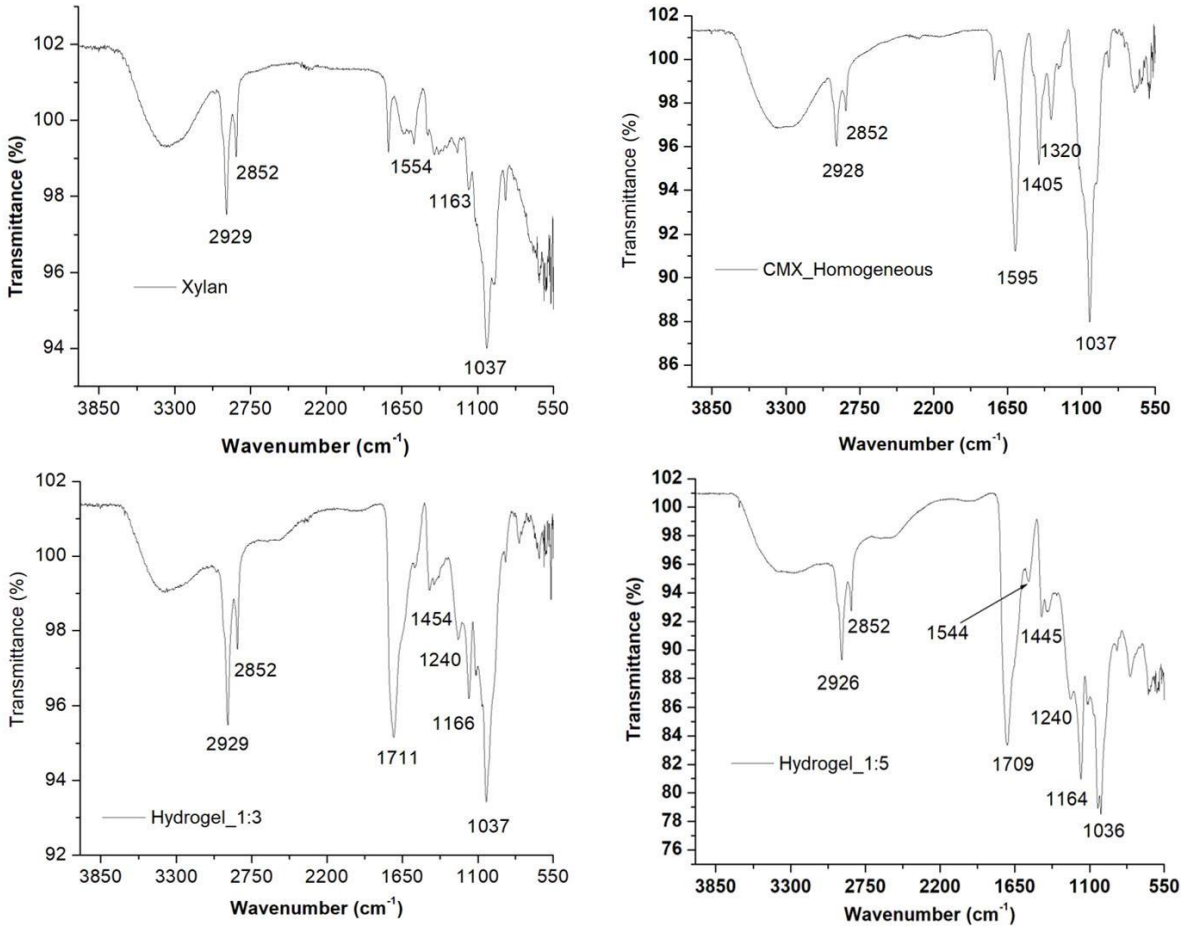


Figure 10. FTIR spectra of xylan, carboxymethyl xylan (CMX), and CMX-based hydrogels prepared with weight-by-weight ratios of 1:3 and 1:5 of CMX-to-AA

The synthesized CMX-based hydrogel spectrum showed the presence of additional new absorption bands at 1709 to 1711 cm^{-1}, indicating C=O stretching modes of the carboxylic group. A sharp absorption band with reduced intensity at 1545 to 1546 cm^{-1} and at 1445 to 1454 cm^{-1} might be attributed to asymmetrical and symmetrical stretching vibration of COO^- respectively, indicating the presence of COO^- groups in the hydrogel network (Peng et al. 2011; Boruah et al. 2014). The characteristic absorption bands at 1240 cm^{-1} and 1164 to 1166 cm^{-1} are assigned to the C-N stretching mode of acrylamide crosslinkers. The obtained results indicated that AA monomers were polymerized onto the CMX backbone, and thus it could be inferred that the CMX-based hydrogels were successfully synthesized by copolymerizing the AA with the CMX backbone (Amin et al. 2012; Rahimi et al. 2012; Lim et al. 2015).

3.11 Study of internal structure

The internal structure of CMX-based hydrogels was characterized using Field Emission Scanning Electron Microscopy (FE-SEM). The FE-SEM micrographs of the cross-sections of the optimized hydrogels are presented in Figs 11 (A) and (B). The micrographs demonstrated the very porous architecture of the synthesized hydrogels containing CMX to AA in a 1:5 weight to weight ratio have an average pore size of 0.52 ± 0.18µm.

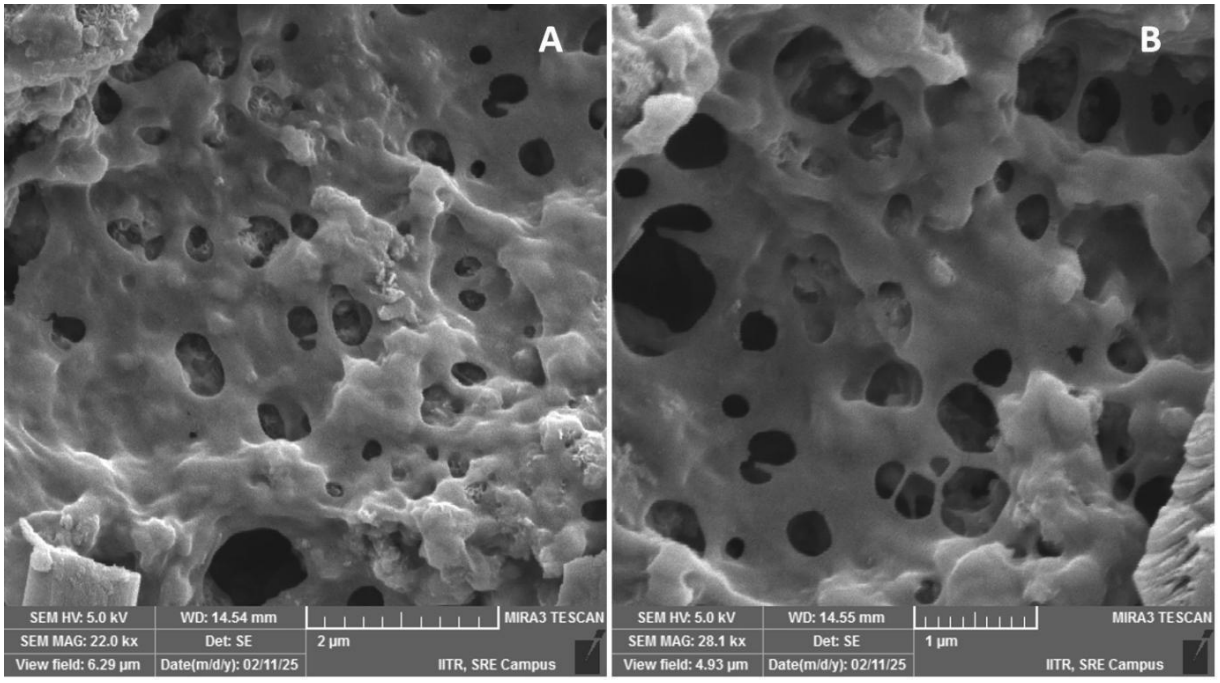


Figure 11. SEM micrographs of hydrogels synthesized with a weight-to-weight ratio of CMX to AA of 1:5.

4. CONCLUSIONS

Green hydrogels based on CMX were synthesized using a free-radical graft copolymerization procedure, utilizing AA monomer crosslinked in CMX solution with MBA as the crosslinking agent and potassium persulfate/sodium sulfite ($K_2S_2O_8/Na_2SO_3$) as the initiator. The FTIR spectroscopy data revealed the effective production of hydrogels. Of all the synthesized hydrogels, the formulation with a 1:5 weight-to-weight ratio of CMX to AA exhibited the highest swelling, exceptional porosity, and optimal in vitro breakdown. The optimized CMX-based hydrogel had commendable mechanical strength for targeted drug delivery with several pores, as illustrated in the micrographs. These results indicate that the hydrogel with a weight-to-weight ratio of CMX to AA of 1:5 can absorb water-soluble medicines more effectively and facilitate the prolonged and efficient release of pharmaceuticals.

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