

Research Article

A Novel Hydrogel Based on Renewable Materials for Agricultural Application

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This study details the design and characterization of a new, biodegradable, and renewable whey/cellulose-based hydrogel (i.e., agricultural hydrogel). This was formulated from cellulose derivatives (carboxymethylcellulose (CMC) and hydroxyethylcellulose (HEC)) and acid whey cross-linked with citric acid, with the aim to obtain an agricultural product with a high swelling capacity to uphold the quality of soil and conserve water resources. With regard to the swelling behaviour of the prepared hydrogels, the authors initially assessed the swelling ratio and capacity for water uptake. Evaluating the chemical structure of the hydrogel and its thermal and viscoelastic properties involved performing Fourier transform infrared spectroscopy, differential scanning calorimetry, thermal gravimetric analysis, and rheological measurement of the hydrogel films. According to preliminary results, sufficient swelling capacity and stiffness were observed in a hydrogel prepared with 3% CMC and HEC, cross-linked with 5% citric acid. Moreover, the kinetics of water uptake revealed a promising capacity that was sustainable after 5 drying and swelling cycles. The results confirmed that the stability of the hydrogel was enhanced by the presence of the citric acid. As a consequence, it is necessary to utilize an appropriate cross-linking concentration and abide by certain conditions to ensure the swelling properties of the prepared hydrogel are sufficient. Further investigation of the topic, especially in relation to applications in soil, could confirm if the whey-cellulose-based hydrogel is actually suitable for agricultural use, thereby contributing to the advancement of sustainable arable farming.

1. Introduction

Agriculture is one of the major consumers of water, accounting for approximately 85% of utilization of available water resources [1]. Recent years have witnessed the ensuing effects of climate change, with water scarcity and associated desertification beginning to impact the agricultural sector. This makes developing new materials for water management and sustainability more pressing than ever before. As a result of such circumstances and emphasis being placed on environmental protection, great interest has been shown in exploring the potential of biodegradable hydrogels for commercial agricultural purposes.

Hydrogels are cross-linked hydrophilic water-soluble polymers capable of absorbing and retaining a large amount

of water, up to a hundred fold the given dry weight, and desorbing under stress [2]. This has led to them being deployed in a wide range of applications, including agriculture [3]. As a conditioner of soil, they increase its capacity for water and nutrient retention, also possibly acting as a slow-release fertilizer [4]. When hydrogels are applied to soil, they improve its permeability, structure, and density, positively affecting the evaporation and infiltration rates of water and may even reduce erosion and water run-off, all to the feasible benefit of crop yield [5, 6].

However, the majority of commercially available hydrogels are nonbiodegradable synthetic polymers based on acrylates, which degrade slowly; hence, they have been deemed potential pollutants of soil [7]. A great deal of recent research has focused on devising polymeric absorbent materials that

are environmentally friendly, founded upon renewable resources as opposed to synthetic hydrogels of petroleum origin [8–10].

The development of a low-cost and environmentally friendly materials based on renewable bioresources (e.g., derivatives from agricultural crops, industrial by-products, or modified lignocellulosic materials) has recently been used in many applications, due to their sustainability and biodegradability [11–13].

With specific regard to the ecological and sustainable characteristics of such a hydrogel for agricultural usage and biodegradation in soil, the authors opted to investigate cellulose derivatives for preparation in this study. Compared to acrylic-based ones, those prepared from cellulose have a good level of stiffness, better absorbency, and high biodegradability [14]. The sodium carboxymethylcellulose (CMCNa) utilized is a water-soluble, biodegradable material, which possesses hydrophilic carboxylate groups in its polymeric backbone. The high absorption, sensitivity to pH, and ionic strength it demonstrates mark it out as an ideal material for preparing hydrogels. When CMCNa is used alone, though, the resultant hydrogels show poor strength, as intramolecular cross-links are formed instead of intermolecular ones [8]. As a consequence, another cellulose derivative—hydroxyethylcellulose (HEC)—was applied in combination with CMCNa to improve such intermolecular cross-linking and stabilize the polymer structure [2, 15]. This type of CMCNa/HEC binary system has been featured in several papers in the literature, wherein swelling properties were reported as similar to conventional acrylate-originating products [8, 16–18].

Acid whey is a by-product resulting from the production of soft cheese, and dairies find it challenging to dispose of due to its low pH and content of high ash and organic acid. It was estimated that global whey production equalled 200 million tonnes in 2016, with a slight rise reported in the years since then [19]. Although several possibilities for utilizing it have been explored, e.g., as whey protein concentrate (WPC) or isolate (WPI) by the food industry [20], a major proportion is discarded as effluent, constituting a form of environmental pollution [21]. Although numerous studies have looked into the possibility of employing WPI or WPC as a core component of hydrogels, there have been no reports on using untreated acid whey for hydrogel synthesis and its resultant potential in agriculture.

It has been found that the concentration of ca 4% of whey protein is sufficient for gel formation at the pH level of the isoelectric point of whey proteins (approx. pH 4.5) [22]. Another option for enhancing hydrogel formation seems to be the use of a polyfunctional carboxylic acid (e.g., citric acid (CA)) for better polymerization of samples [20].

In this study, CA was used as a nontoxic cross-linking agent. At sufficiently high temperatures, CA reacts with the high hydrophilic carboxylic groups of the cellulose, caused by an esterification mechanism founded on the formation of anhydride intermediate [16]. Hydrogels cross-linked by CA possess stiffness that proves adequate enough to maintain their shape and higher swelling ratio [16]. For these reasons, it was believed that CA would make a suitable alternative to acrylic acid.

The authors detail in this work the preparation and properties of a novel biodegradable hydrogel based on renewable materials for potential agricultural application. The hydrogel was formulated from low-cost, widely available materials, created by simple manufacturing processes, in order to reduce production costs and avoid risk of a toxic intermediate arising during the procedure. Acid whey, a by-product of the dairy industry, was employed as a dissolving medium for the biodegradable superabsorbent polymers, these being cellulose derivatives (CMCNa and HEC) which were mixed with CA to obtain a cross-linking reaction with the aim of forming the subsequent hydrogel. Research focused on investigating the potential of the novel hydrogel, especially its swelling capabilities, so as to heighten the capacity for water retention in soil.

2. Materials and Methods

2.1. Materials. Carboxymethylcellulose sodium salt (CMCNa) (Blanose 7HOF, with MW 7×10^5 Da, DS = 0.7, viscosity 1000–2800 cp (1%, 25°C)), and hydroxyethylcellulose (HEC) (Natrosol 250 HR, with MW 106 Da, MS = 2.5, viscosity 1500–2500 cp (1%, 25°C)), purchased from Ashland, USA, were utilized for the hydrogel preparation. Acid whey, a by-product of the manufacture of cheese, was sourced from a small, organic dairy farm in the Czech Republic and employed as the solution for such preparation. Citric acid (CA) in anhydrous form (Sigma-Aldrich, Milano, Italy) was applied as a cross-linking agent. Evaluating swelling properties at various pH levels involved adjusting the saline solution by adding drops of 1M NaOH solution.

2.2. Hydrogel Preparation. The hydrogel samples were prepared according to the procedure detailed below, as described in the literature [16, 18] albeit with slight modification. The composition of the samples is given in Table 1. Initially, a mixture of the CMCNa and HEC at the total polymer concentration of 3% wt, at a weight ratio equal to 3:1, was dissolved in the acid whey (alternative to distilled water commonly used for hydrogel preparation) by gentle stirring on a mixer (RW 20 digital, IKA) at room temperature until a homogeneous solution was formed that showed a significant increase in viscosity (16–24 h). Afterwards, the CA was added to the solution at different concentrations (3, 5, and 10% wt) to obtain samples with various degrees of cross-linking. The hydrogel samples were cast as a 10 mm thick hydrogel in Petri dishes. The cross-linking reaction was achieved during the following drying cycle of the hydrogel samples at 60°C for 24 hours. Xerogel, a dried form of the hydrogel, was procured for subsequent characterization. In order to evaluate the swelling behaviour of the CMCNa/HEC whey hydrogel, the hydrogel samples containing only CMCNa or HEC cross-linked with CA were prepared according to the same procedure described above. To evaluate the effect of cross-linking temperature on the swelling behaviour of hydrogel and dependency of cross-linking temperature on viscoelastic properties of swollen hydrogel, the samples of different cross-linking temperatures (30, 50, 60, 70, 80, and 90°C) were also prepared.

TABLE 1: Composition of the hydrogel samples.

Sample designation	Type and concentration of cellulose (wt %)	Concentration of CA (wt %)
H0		0
H3	3% CMCNa/HEC	3
H5	3:1	5
H10		10
CMC	3% CMCNa	3, 5, and 10
HEC	3% HEC	3, 5, and 10

2.3. Characterization of the Whey/Cellulose Hydrogels

2.3.1. Swelling Ratio and Equilibrium Water Uptake. Evaluating the capacity for water absorption of the cellulose-based hydrogel samples prepared from acid whey involved gauging their swelling ratio (SR) and swelling kinetics at certain time intervals via the gravimetric method. In order to compare swelling properties of such hydrogels and commonly available hydrogels based on water, the cellulose-based hydrogel samples manufactured from distilled water were also prepared using the same method. An analytical balance was employed to measure weight. The SR was determined by weighing the samples before and after immersion in distilled water at room temperature for 24 h according to the following procedure. The mass of the dried hydrogel samples was first weighted (± 3 g) and then moved to the container with 300 ml distilled water at temperature 20–22°C and immersed for 24 hours without stirring. Swelling kinetics were quantified by periodically removing the hydrogels from the container at the intervals of 5, 10, 15, 20, 30, 60, 180, 240, and 1440 minutes, blotting them on filter paper to remove excess water and weighing them immediately to observe equilibrium water uptake. The SR (the water content of the swollen hydrogel, in per cent) was calculated via the following equation:

$$SR = \left(\frac{W_s - W_d}{W_d} \right) \times 100, \quad (1)$$

where W_s and W_d are the masses of the swollen and dried samples (g), respectively. All the samples were analysed minimally in triplicate. The swelling behaviour of the prepared samples was gauged in different media by immersing the samples in distilled water (DW), acidic (pH 2.5 and 4.5), neutral (pH 7.2), and alkaline (pH 8.0 and 10.0) media.

2.3.2. Reswelling Capacity. The SR was also calculated for five drying and swelling cycles of each sample to determine hydrogel reversibility and the possibility of water reabsorption (repetition of the swelling–deswelling–reswelling process). The act of determining the water reswelling kinetics (RSW) required that the hydrogel samples were dried repeatedly in a well-ventilated oven at 30°C for 24 h after each swelling cycle. The RSW of the swollen hydrogel was then gauged according to the equation for SR given above. The

drying–swelling process constituted a single reswelling cycle, and this was repeated five times for every hydrogel sample.

2.3.3. Hydrogel Solubility in Water. In order to evaluate the solubility (S , in percent) of the hydrogels during the period of swelling in water, the samples were weighed (g) after each cycle of drying (at 30°C for 24 hours); the equation for this was as follows:

$$S = \left[\frac{\text{Initial dry weight}}{\text{Initial dry weight} - \text{final dry weight}} \right] \times 100. \quad (2)$$

2.3.4. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy. Infrared spectra for the CMC, HEC, CA and cross-linked hydrogels were recorded on a Thermo Nicolet 6700 spectrometer via the ATR technique, equipped with a diamond crystal, which was set to the resolution of 2 cm^{-1} and range of absorbance of $400\text{--}4000 \text{ cm}^{-1}$. The samples of xerogel had been ground in a powder prior to such measurement.

2.3.5. Viscoelastic and Thermal Properties. The viscoelastic properties of the hydrogels were characterized by rheological analysis (frequency sweep and strain sweep tests). Rheological measurements were carried out in triplicate with fully water-swollen (24 h) hydrogel particles (of diameter $150\text{--}200 \mu\text{m}$) on a rotational rheometer (Physica MCR 502, Anton Paar GmbH, Austria) set up with a parallel-plate geometry (PP 20/MRD/TI/S, $d = 20 \text{ mm}$) at the temperature of 25°C. The prepared hydrogels were accurately placed between the plates. The viscoelastic properties of the prepared samples were studied by carrying out oscillation tests. The strain sweep tests were conducted at $\omega = 1.0 \text{ rad/s}$ at the strain amplitude of 0.01% in order to ensure all data fell within the linear viscoelastic region (LVR). Frequency sweep tests were performed in the frequency range of 0.1–40 Hz at the abovementioned strain setting.

The researchers performed differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) to evaluate the thermal properties of the hydrogels. A differential scanning calorimeter (a Mettler Toledo DSC1 Star unit) was employed for thermal analysis to discern the differences in heat between the hydrogels. The samples were heated from 30°C to 200°C in an inert nitrogen atmosphere at the flow rate of 10 ml/min. The thermal stability of the hydrogels was studied by TGA on a Mettler-Toledo TGA/SDTA 851e instrument under the condition of nitrogen flow (10 ml/min^{-1}). A heating rate of $10^\circ\text{C min}^{-1}$ was applied at intervals, the temperatures ranging between 25°C and 500°C.

3. Results and Discussion

Based on previous studies [16–18, 23], our hydrogel was prepared by combining CMCNa and HEC at the ratio of 3:1 (3% wt) in acid whey solution, cross-linked by CA, so as to obtain a biodegradable hydrogel with excellent swelling capacity. The swelling–deswelling ability of the hydrogel is crucial to the given agricultural applications of soil conditioning and controlled nutrient release. The swelling behaviour of

each prepared hydrogel was investigated first. To this end, the swelling ratio, water uptake capacity over time, and repeated absorption were studied, as well as the effect of pH, cross-linking temperature, and CA content on such swelling; the latter being carried out with regard to the water holding capacity and viscoelastic properties of the hydrogel. The swelling parameters of the whey/cellulose hydrogels are summarized in graphs below (Figures 1–7).

The authors determined the swelling capacity of the polymers by preparing hydrogels solely based on CMCNa and HEC, with adherence to the same procedure, using acid whey as the polymeric solution. The results given in Figure 1 reveal that the combined CMCNa/HEC hydrogel showed a higher swelling capacity than samples containing just CMCNa or HEC, these possessing the same CA concentration, after 24 hours of swelling in distilled water. The HEC-based hydrogel was less stable and demonstrated a lower potential for absorption once CA had been introduced. The explanation for this lies in its low cross-linking capability, brought about through a higher degree of substitution (fewer -OH groups) than CMCNa [18]. As is well-known, CMCNa is a polyelectrolyte compound that exhibits sensitivity to pH and ionic strength. CMCNa enhances the swelling capacity of a hydrogel as a consequence of the Gibbs-Donnan effect, which promotes osmotic pressure, thereby forcing water to enter the hydrogel and inhibiting any rise in the ionic strength of the external solution [24]. However, poor cross-linking efficiency has been reported when only CMCNa is utilized, due to electrostatic repulsion between the charged macromolecules of polyelectrolyte chains [16]. Therefore, HEC, which promotes the formation of intermolecular rather than intramolecular cross-links in hydrogels, was added to the whey/cellulose solution to stabilize the macromolecules in a three-dimensional polymer network [15]. Such swelling capacity can also be enhanced by increasing the content of CMCNa. This explains why the hydrogels prepared with a combination of CMCNa/HEC at the ratio of 3:1 demonstrated a higher swelling capacity and greater structural stability than the purely cellulose-based derivatives.

As confirmed elsewhere, swelling behaviour is not only dependent on the ratio of the polymer but it can also be modified by varying the amount of cross-linking agents [2, 8, 25]. Herein, the cross-linking reaction was achieved via esterification of polyfunctional carboxylic citric acid with cellulosic hydroxyl groups [26]. The results of the swelling tests revealed that CA concentration affected the amount of water absorbed during the period of swelling (Figure 2). When a heightened concentration of CA was present in the polymeric solution (causing rise in cross-linking density), lower uptake of water was observed (swelling capacity decreased). It was shown that the hydrogel samples without CA (the blank) and those with a low concentration of CA (H3) were insufficiently formed due to their limited extent of cross-linking, in turn leading to dissociation of the highly hydrophilic carboxylic group of CMCNa, thus to increase in equilibrium swelling and the loss of integrity of the hydrogel. In contrast, though, high concentrations of CA (H10) promoted a rise in carboxyl content; hence, hydrogels of greater solidity were formed, albeit with reduced swelling capacity; both outcomes

were potentially due to the increase in cross-linking density, this altering the physical state of the polymer chains and reducing the free volume of the hydrogel structure [9, 27]. These findings are in accordance with a study by Demitri et al. [16], wherein the swelling ratio of the hydrogel was also dependent on CA concentration, and an optimal degree of swelling (about 900%) was achieved when such concentration was low (3.75%). A similar extent of swelling (approximately 1000%) was reported in another paper by hydrogels prepared from CMCNa and HEC cross-linked by 5.75% *w/w* CA [18]. The swelling capacities of the said cellulose-based hydrogels in the literature are comparable with the novel cellulose/whey-based hydrogel cross-linked by CA detailed herein. Although a higher absorption capacity was observed here by the authors for the samples prepared from water, the sample with whey showed similar values at the CA concentration of 5% wt (see Figure 2). Moreover, the novel hydrogels displayed better consistency and stability than those prepared from water, while the sample denoted as H5 even exhibited a higher swelling capacity (almost 1700%) than established (acrylic-based) hydrogels manufactured on the basis of deionized water [24, 28]. These findings demonstrate that the novel biodegradable hydrogels exhibit significantly greater swelling capacities than commercially available cellulose-based superabsorbent materials with swelling ratios of 380–680% [29, 30].

Similar studies to this have found that cellulose-based hydrogels prepared by various cross-linking agents showed a different degree of swelling compared to hydrogels synthesized by CA. For example, cellulose-based hydrogels prepared by cross-linking with succinic anhydride absorbed 400 times the dry weight of the gel [31]. Seki et al. [32] synthesized a CMC/HEC hydrogel with fumaric acid and malic acid across a range of concentrations. Their hydrogels with malic acid at the concentration of 15% possessed a higher absorption capacity (water uptake of up to 500%) than those based on fumaric acid; this may have been due to structural differences in the -OH functional group of the malic acid [32]. Regarding experiments with synthetic cross-linking agents, Alam et al. [28] prepared a CMC-based hydrogel cross-linked by epichlorohydrin with an equilibrium water uptake of 725 times the dry weight of the hydrogel. In a study by Sannino et al. [24], hydrogel samples were chemically cross-linked with divinyl sulphone, the same exhibiting high swelling values (around 1000%). This combined evidence suggests that CA has the potential to function as a cross-linking agent that promotes a comparable or greater swelling capacity of the cross-linked hydrogel.

A great number of studies have reported on utilizing whey protein (WPI and WPC) or its components, e.g., β -lactoglobulin and bovine serum albumin, in the preparation of protein-based hydrogels and their possible application in the food industry [22] or as delivery systems for drugs or nutrients [33, 34]. Although lower values of the swelling ratio for such WPC and WPI hydrogels have been reported (10–100%) [22, 34], adding in an absorbent material, such as polysaccharide (e.g., chitosan, CMC), might increase the subsequent swelling ratio by an order of magnitude (100–550%) [35]. Although sweet whey with a high protein content is presently employed in the production of WPI and WPC, low-protein

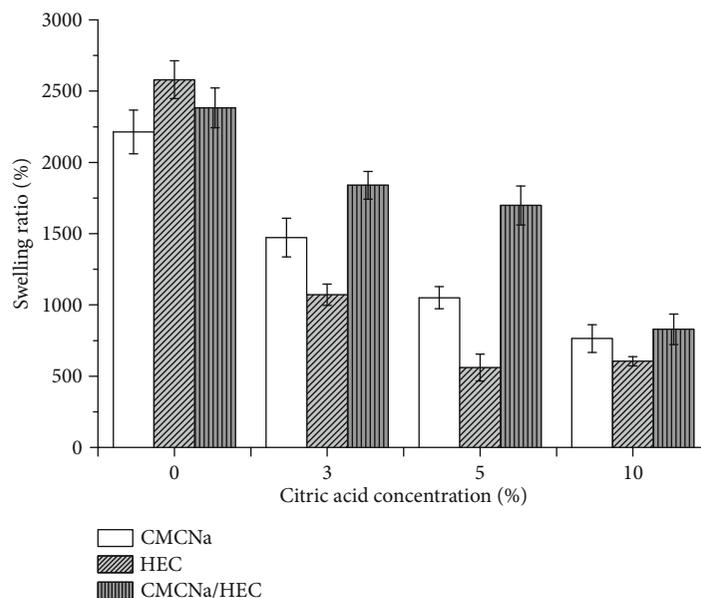


FIGURE 1: Hydrogel swelling capacity (%) of CMCNa, HEC, and CMCNa/HEC whey hydrogel (3% wt) in distilled water. The bar graph shows the dependence of water absorption (g/g) on CA concentration (3, 5, and 10% wt).

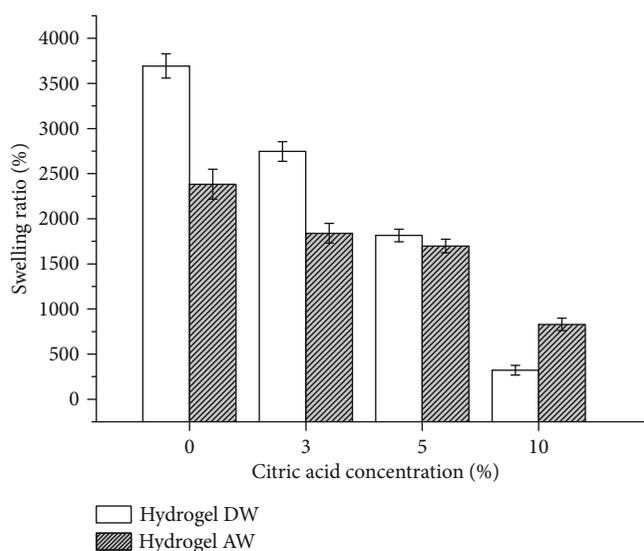


FIGURE 2: The swelling ratio (%) of the CMCNa/HEC hydrogels (3% wt) manufactured from water (hydrogel DW) and acid whey (hydrogel AW), cross-linked by different concentrations of CA.

acid whey could also constitute a valuable compound for hydrogel synthesis with potential application in agriculture. It has been claimed that assuring the appropriate composition and concentration of proteins (at least 4%) is a requirement for successful gel formation [22]. However, an additional parameter, such as pH, is also a necessity [34]. Herein, it was found that at approximate pH of the isoelectric point of the whey protein (pH 4.5), a low concentration of protein (ca 0.5%) was sufficient for gel formation of the cellulose derivatives. In addition, the CA enhanced the polymerization of the hydrogels prepared from acid whey. Based on these preliminary results, the CMCNa/HEC hydrogel cross-linked by 5% of CA in the acid whey solution showed the best swelling

values as well as viscoelastic properties that ensure good reswelling ability. Hydrogels based on acid whey show promise and are worthy of further investigation with the aim of optimizing production and characterization.

The low-protein acid whey replaced the distilled water commonly used to synthesize hydrogels. The swelling results of the whey/cellulose-based hydrogels showed high swelling capacities (1000-1700%), comparable with other cellulose-based superabsorbent hydrogels manufactured from water. As the findings of this study reveal, combining acid whey with the cellulose derivatives CMCNa and HEC results in excellent swelling properties, hence creating a possibility to effectively utilize this waste product of the dairy industry.

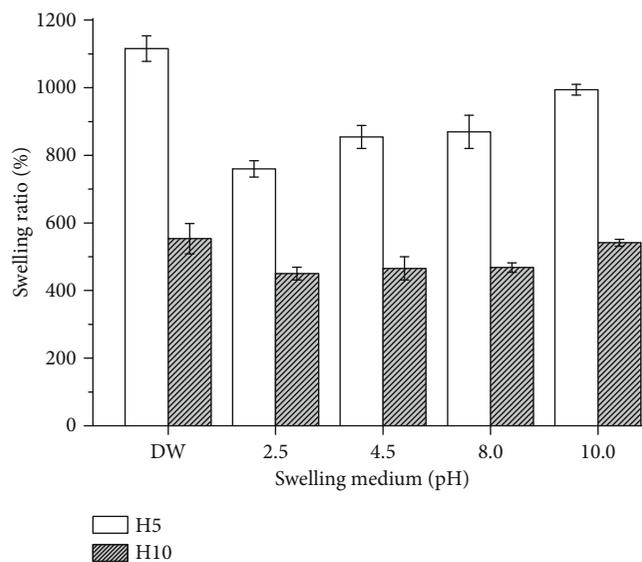


FIGURE 3: Effect of different media and pH on hydrogel swelling capacity (%) after 24 h of swelling.

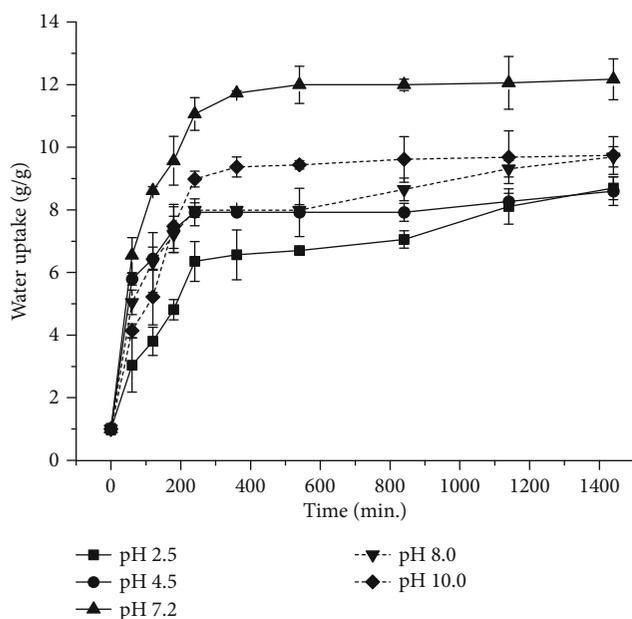


FIGURE 4: Effect of acidic (pH 2.5 and 4.5) neutral (pH 7.2) and saline (pH 8.0 and 10.0) solutions on the water uptake of hydrogel H5 (g/g dry weight) after 24 h of swelling.

Swelling media of different pH were employed in this study to verify the effect of pH on the swelling capacity of the cellulose/whey hydrogel. Figure 3 reveals that both of the tested hydrogel samples (H5 and H10) reached their maximum swelling capacities (%) after they had been immersed in the distilled water (DW) (1115 and 554) and saline solution at pH 10.0 (994 and 541), respectively. Conversely, a considerable decrease in swelling capacity occurred in the acidic medium at pH 2.5. These changes in swelling behaviour were caused by ionization of the carboxyl groups [36]. At pH values higher than the pK_a of the carboxylic groups (pK_a 4–5), the carboxylic acid groups became depro-

tonated and electrostatic repulsive forces between the negatively charged sites (COO^-) enhanced water uptake capability. This contrasted with low pH values, which caused most carboxylate anions to protonate (the ionized COO^- groups became $COOH$ groups); thus, such repulsive forces were eliminated and the degree of swelling diminished as a consequence [32].

The influence of the absorbing medium on swelling kinetics was evaluated by measuring the water uptake of the hydrogel sample at certain time intervals. Variations in the swelling behaviour of hydrogel H5 are given in Figure 4. The highest water uptake by H5 was reached in distilled water (pH 7.2), followed by the saline media (pH 10.0 and pH 8.0) and acidic media (pH 4.5 and 2.5) during the first hydration cycle. This dependence on the general decrease in pH might have stemmed from dissociation of the carboxylic groups. Since one of the polymers was pH-sensitive (CMCNa), the hydrogels demonstrated good swelling at neutral pH but lower swelling ratios in the acidic media. This was caused by deprotonation of the carboxylic acid groups at higher pH levels ($pH > 4$) [37]. In acidic media, though, the majority of the carboxylate anions were protonated while electrostatic repulsion was restricted amongst the carboxylate groups, thereby reducing the water uptake of the hydrogels [37]. Other instances of physical cross-linking, e.g., hydrogen-bonding interactions between the carboxylate groups, may also have contributed to the reduction in swelling capacity [32]. Seki et al. [32] observed similar swelling behaviour in a CMC/HEC hydrogel synthesized via fumaric acid and maleic acid; they observed greater water uptake values in hydrogels at higher pH values (7.4). In a study by Bukhari et al. [36], the maximal swelling of an acrylic acid/gelatin-based hydrogel was also obtained at pH 7.5.

On the basis of the results described, it is evident that efficient swelling of the hydrogel can be attained when the pK_a of the solution exceeds that of the carboxylic group. As Figure 4 shows, the optimum pH for swelling of the

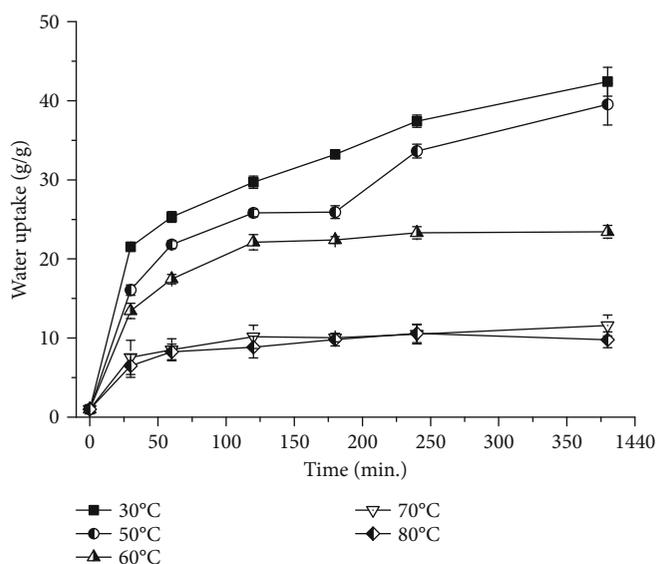


FIGURE 5: Effect of cross-linking temperature on the swelling behaviour of hydrogel H5 over time. The samples were cross-linked for 24 h at various temperatures. Water uptake (g/g) was measured by weighing the samples immersed in distilled water, at room temperature for 24 hours.

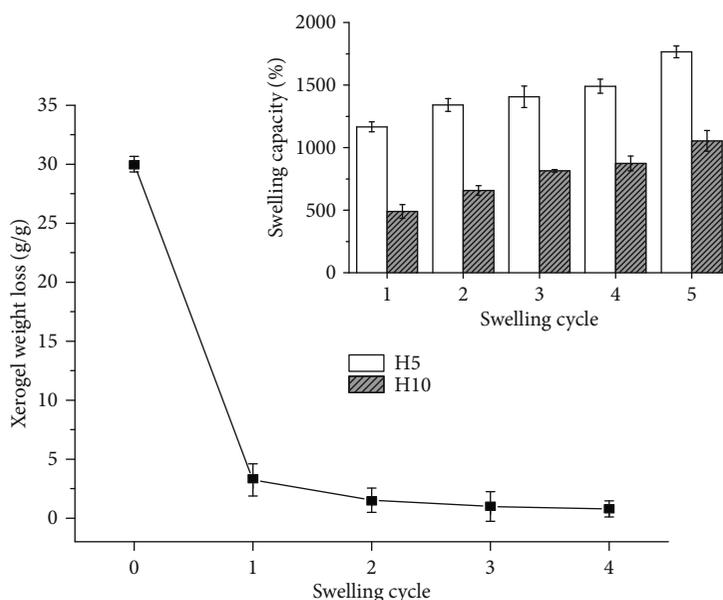


FIGURE 6: Water absorption capacity of the hydrogels following five consecutive reswelling cycles and loss in weight (solubility of xerogel) after each drying process.

whey/cellulose hydrogel is approximately 7.2. It should also be pointed out that if the solution or another external environment possesses high ionic strength, the swelling behaviour of the hydrogel may be influenced. It is clear that further investigation on the effects of the chemical compounds of media on such swelling is advisable.

The degree of hydrogel swelling also depends on the cross-linking temperature detailed in Figure 5. The best ability for absorption was seen for the hydrogel cross-linked at a low temperature (30°C and 50°C, respectively), since poor cross-linked hydrogels are formed at a lower temperature.

At an elevated temperature, the hydrogels become firmer and tough with a brown-tinted film, with a consequent reduction in swelling capacity. This reduction might stem from the formation of strong hydrogen-bond interactions between the polysaccharides and CA during the cross-linking reaction, giving rise to strong cross-links, shrinkage of the polymer network and decrease in swelling capacity. As for whey/cellulose-based hydrogels, a high temperature for an extended period of sample drying (i.e., a cross-linking reaction) might contribute to the formation of a greater amount of insoluble proteins, thereby lowering

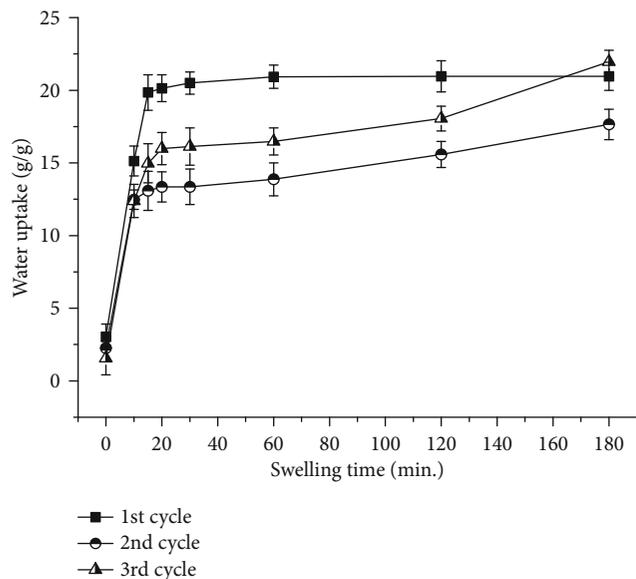


FIGURE 7: Water absorption capacity of hydrogel sample H5 following three consecutive swelling cycles, preceded by a drying process for each.

swelling capacity. Optimal hydrogel swelling was observed upon cross-linking at the temperature of 60°C.

Analysing the curves for water absorption kinetics revealed that the maximum increase in water uptake was observed at 60 and 120 minutes for samples heated at a low temperature (30, 50 and 60°C). A contrary, slight rise in swelling capacity until equilibrium was observed in samples cross-linked at a high temperature (70, 80, and 90°C). This confirms that a high temperature and a long drying process may lead to a decrease in swelling properties.

The authors also investigated the effect of swelling-drying cycles on the swelling capacity and solubility of the hydrogels. Their water absorption capacity following five consecutive reswelling cycles is depicted in Figure 6, wherein the swelling ratio increases in line with the rise in the number of such cycles. The increase in the solubility of the xerogels is also visible.

The curves for swelling kinetics are given in Figure 7 and show fast water uptake in every swelling cycle. Such swelling in distilled water progressed rapidly, reaching 20 g water/g of dried hydrogel in 15 minutes during the first swelling cycle. This contrasts with the second cycle, when the water absorption rate slowed down; equilibrium water uptake was attained at 24 h. The probable cause was the formation of hydrogen bonds between $-\text{COOH}$ and $-\text{OH}$ groups during the first cycle [28]. However, it was in the third cycle that the highest water uptake was observed, with a slight increase in absorption in 24 h of the swelling experiment. This reswelling ability significantly outperforms any cellulosic superabsorbent material reported in the literature [28].

FTIR analyses were carried out for the CMCNa and HEC polymers and hydrogel CMCNa/HEC (at the weight ratio of 3:1, cross-linked by 5% CA), in order to confirm different substitutions of cellulose and the formation of ester bonds. So as to evaluate interaction between the CA and cellulose

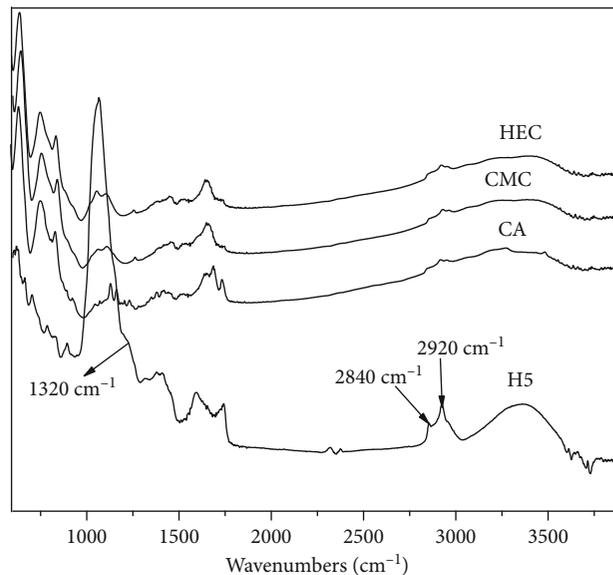


FIGURE 8: FTIR spectra for the CMCNa and HEC polymers, CA, and the hydrogel (H5) prepared from CMCNa and HEC (3% wt) (at the weight ratio of 3:1 with 5% concentration of CA).

derivatives, characteristic bands for the CA were derived. Figures 8 and 9 contain the FTIR spectrum for the hydrogels and its compounds.

Figure 8 depicts the FTIR-ATR spectra for CMCNa, HEC, CA, and the cross-linked hydrogel to confirm that cross-linking reactions had occurred. The spectrum for CA showed a broad peak at 3000-3500 cm^{-1} attributed to the stretching vibration of $-\text{OH}$ and a sharp peak at 1750 cm^{-1} due to the stretch of hydrogen-bonded $\text{C}=\text{O}$. The presence of this band indicates interaction between the CA and polymer [38] as clearly visible in Figure 9. The reaction of the polyfunctional carboxylic acid with the cellulose derivatives came about through fixation of the carboxylic acid via esterification with the cellulose hydroxyl group. Note that further subsequent reaction by esterification with another cellulose hydroxyl results in cross-linking between the cellulose chains [26]. Broad peaks at 3500 to 3000 cm^{-1} are seen in the spectrum of CMCNa, also due to $-\text{OH}$ stretching. This also confirmed the presence of COOH groups in the cellulose derivative and hydrogel. The band at 2920 and 2850 cm^{-1} was due to C-H stretching vibration. The strong absorption band encompassing 1590 cm^{-1} and 2850 cm^{-1} was due to stretching of the COO^- group, respectively. The FTIR spectrum for the hydrogel showed additional peaks at 1710 and 1590 cm^{-1} due to the carbonyl band of free carboxylic acid groups and the carbonyl band of ester formed during cross-linking. However, this peak was also present in the non-cross-linked sample, due to the presence of the CMCNa which contained free $-\text{COOH}$ groups. However, a peak at 1710 cm^{-1} was slightly shifted to a region of higher wavelengths (1742 cm^{-1}). The characteristic absorption bands of CMCNa at 1050-1100 cm^{-1} , related to the C-O stretching vibration of the alcoholic group, were obviously weakened after the cross-linking reaction. For HEC, the typical peak at 3248-3540 cm^{-1} assigned to the OH range indicated

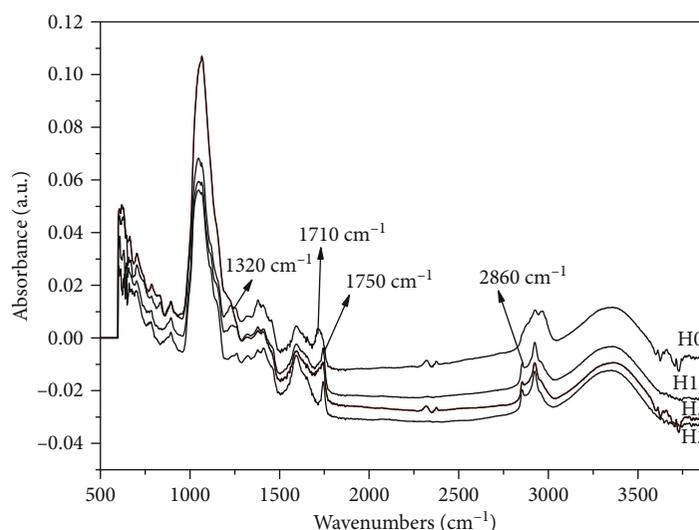


FIGURE 9: FTIR spectra for hydrogels prepared from CMCNa and HEC (3% wt) at the weight ratio 3 : 1 at different concentrations of CA.

intermolecular and intramolecular bonds. In addition, bands at 1354 cm^{-1} and 1646 cm^{-1} , arising through OH-stretching vibration, were observed. Similar characteristic absorption peaks have been seen in the literature for CMC, a CMC-ECH cross-linked hydrogel [28] and CA cross-linked CMC-based composite hydrogel films [8].

The cross-linked hydrogel possessed two additional peaks at 2840 cm^{-1} , as a consequence of the increased number of O-H stretching vibrations, with a sharp, intense peak caused by the formation of ether groups. The band at 2920 cm^{-1} was due to C-H stretching of the $-\text{CH}_2$ and $-\text{CH}_3$ groups and is characteristic of both cross-linked and non-cross-linked samples. A peak also appears at 1320 cm^{-1} , related to the presence and bending vibrations of $-\text{CH}_2-\text{O}-\text{CH}$ groups. The ATR-FTIR spectrum for the hydrogel film showed additional peaks at 1710 to 1730 cm^{-1} , attributed to the carbonyl band of free carboxylic acid groups and the carbonyl band of ester (CA also has a peak at 1690 cm^{-1} pertaining to C=O stretch, but this splits into 2 separate peaks in neighbouring regions upon cross-linking) as well as to asymmetric stretching of the COO^- group at 1590 cm^{-1} .

The thermodynamic behaviour of differently cross-linked hydrogel samples was characterized by DSC to confirm the degree of cross-linking and define dependence on hydrogel composition.

Figure 10 shows the heating run of the whey/cellulose-based hydrogel cross-linked by CA up to the temperature of 200°C . The heat required for melting the cross-linked hydrogel sample was 138.5 J/g for H0, 151 J/g for H5, and 163 J/g for H10, respectively. A large endothermic peak at around 100°C was associated with the evaporation of water produced by the anhydriification process.

The DSC thermogram curves also revealed differences in the heat flow of the analysed samples, indicating the formation of ester cross-links, in agreement with previous reports [8, 16, 32]. The peaks that start at 184°C for H0, 171°C for H5, and 161°C for H10, respectively, correspond to the thermal decomposition of the polymer. Peak values are reduced

for samples with at a higher amount of cross-linker through the involvement of hydrophilic hydroxyl side-groups in the cross-linking reaction. Concurrently (as mentioned earlier), their ability to bind water molecules is reduced. The results obtained by DSC show that the thermal behaviour of the hydrogels depends on the degree of cross-linking of the polymer tested. The thermogram also disclosed that the hydrogel samples were stable up to 200°C . It is worth noting that their thermal stability may be enhanced, for example, by incorporating specific inorganic nanoparticles during hydrogel synthesis [37].

Thermogravimetric analysis was performed to gauge the thermal stability of the prepared hydrogels. Figure 11 shows the TGA curves and derivative TGA curves for the hydrogels containing different concentrations of the cross-linking agent and non-cross-linked hydrogel. In the literature, the maximum degradation temperatures for CMCNa and HEC have been reported as 284°C and 280°C [32]. In this study, the CMCNa/HEC cross-linked hydrogel possessed a higher thermal stability than the corresponding cellulose precursors. The greatest degree of degradation for the cross-linked hydrogel was established at $300\text{--}320^\circ\text{C}$; all volatiles and easily degradable organic materials were removed at this temperature, confirming that the cross-linked hydrogels possessed heightened thermal stability. The cross-linked samples showed about 24% of disintegration at the temperature of 190°C in all cases, correlating with the degradation temperature of CMCNa [32, 39], while only two stages were detected for the cross-linked hydrogels. The first stage of mass loss may be attributable to the evaporation of the bound water [39], whereas the second stage of decomposition could have been due to degradation of the material (related to decomposition of the cross-linker) [15]. It was discerned that the hydrogels prepared in the presence of the cross-linker exhibited a lower thermal decomposition temperature than the initial composition without the cross-linking agent. This drop in thermal stability was a consequence of the decrease in the enthalpy of the system after cross-linking had occurred

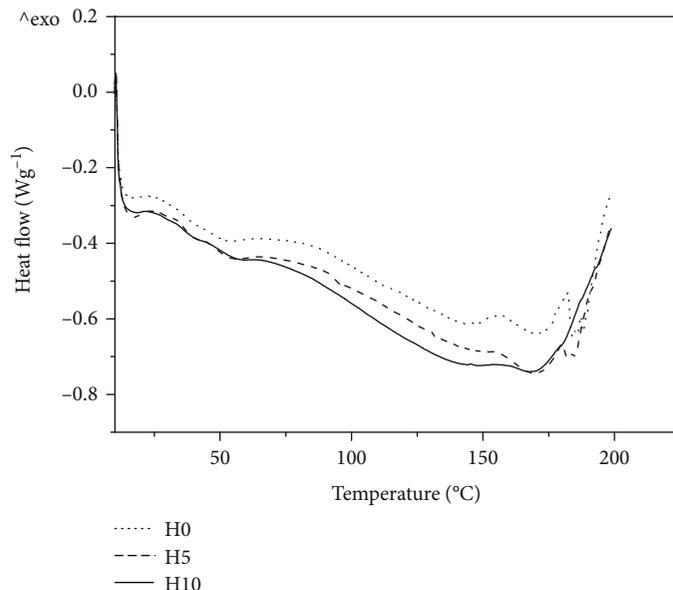


FIGURE 10: DSC thermogram of hydrogels cross-linked by 5% CA (H5), 10% CA (H10), and non-cross-linked hydrogel (H0).

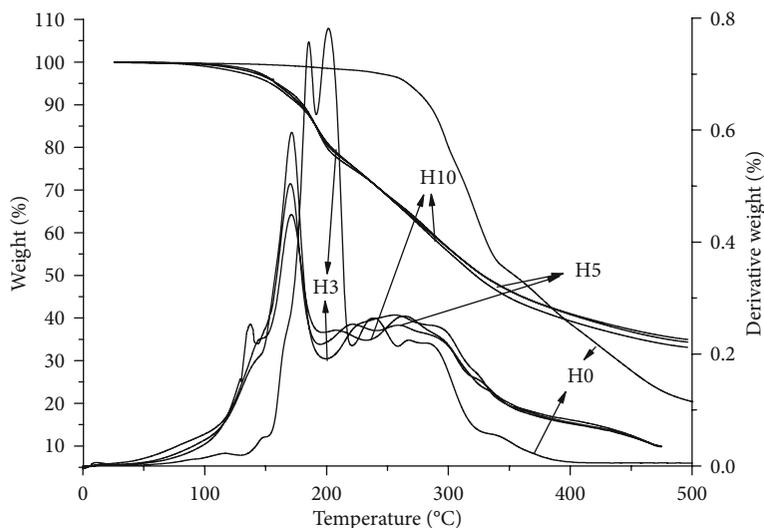


FIGURE 11: TGA thermogram of hydrogel samples with different concentrations of CA (H3, H5 and H10) and the non-cross-linked hydrogel (H0).

[15]. Derivative TGA curves for the H5 and H10 cross-linked samples showed two temperature peaks corresponding to the first and second stages of decomposition. The cross-linked hydrogels exhibited an extra degradation peak at 130°C, potentially caused by evaporation of residual adsorbed water that had been retained in the hydrogels. This data confirms the superior water absorption capacity of the cross-linked hydrogels in comparison with the initial composition.

The rheological data given in Figure 12 details the dependency on viscosity for the variously cross-linked H5 samples at certain frequencies after one hour of swelling. Viscosity helps define the ability of a hydrogel to resist deformation in response to stress. The graph shows that viscosity

decreased as frequency rose, possibly because raising frequency brings about a drop in the relaxation time and favours elastic properties over viscous ones, as minimal time is available for energy to dissipate during the experiment. Despite this, the hydrogel tested tended to retain its elasticity at lower load frequencies [40].

The figure also reveals that the points marking viscosity for the samples cross-linked at 30°C and 50°C followed a very similar trend at lower positions than the others, due to the similarity in the cross-linked structures of the samples. Moreover, at 60°C, 70°C, and 80°C, the samples showed marginally analogous viscosity, as a consequence of their highly cross-linked structures. By reason of the viscosity and extent of

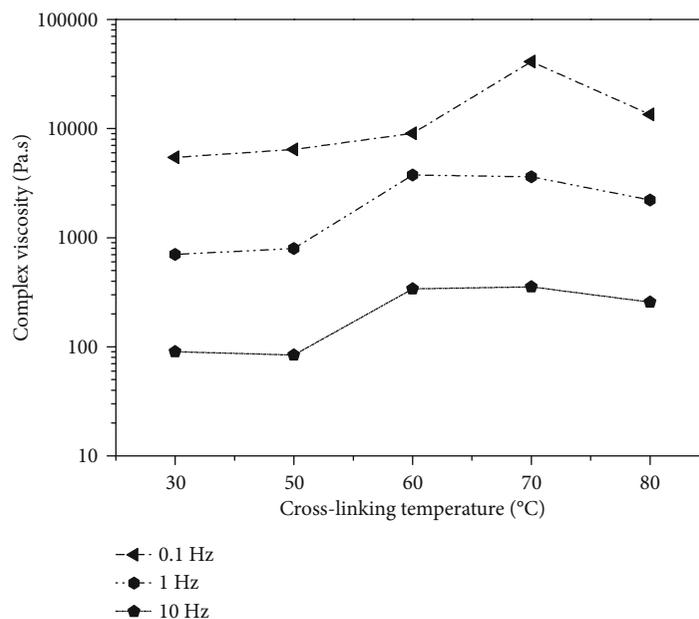


FIGURE 12: Dependency of complex viscosity on curing temperature for the H5 samples differently cross-linked at certain frequencies after one hour of swelling.

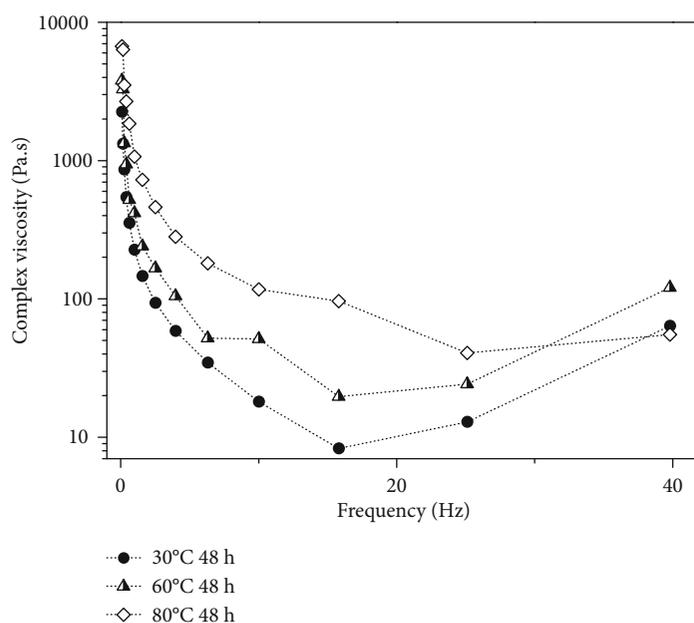


FIGURE 13: Dependency of complex viscosity on frequency for the H5 samples cross-linked at 30 °C, 60 °C, and 80 °C after 48 hours of swelling.

cross-linking demonstrated, and taking temperature into consideration, the sample cross-linked at 60 °C was chosen as the most appropriate sample.

Figure 13 demonstrates the dependency of complex viscosity on frequency for the H5 hydrogel samples cross-linked at different temperatures (30 °C, 60 °C, and 80 °C) after 48 hours of swelling. The lowest viscosity was observed for the one at 30 °C, due to insufficient cross-linking, whereas the highest viscosity was recorded for that of 80 °C since it exhibited high cross-linking. It is evident, therefore, that the

temperature at which cross-linking occurs is an important aspect in forming a hydrogel and shaping its rheological properties. In addition, it shows that a hydrogel sample cross-linked at a higher temperature is more stable after a long period of swelling.

Bearing practicality in mind, the behaviour of hydrogels placed under oscillation load after a certain interval of swelling is of immense importance. A higher storage modulus denotes greater solidity. A hydrogel with a high storage modulus is characterized by superior strength and rigidity [41].

TABLE 2: Dependence of storage modulus on cross-linking temperature after swelling intervals of 1, 24, and 48 hours.

Cross-linking temperature	Storage modulus [Pa]		
	1 hour	24 hours	48 hours
30°C	4958.33	5164.17	1380
60°C	21 016.67	11 228.33	2581.67
80°C	14 816.67	10 120	7110

Table 2 depicts the dependency of storage modulus on cross-linking temperature after swelling intervals of 1, 24, and 48 hours. Storage modulus was the highest for the hydrogel cross-linked at the temperature of 60°C; notably, this sample with the greatest elastic moduli and water absorption experienced a dramatically fall in storage modulus. By way of explanation, when the degree of cross-linking is greater, material strength is improved by the higher storage modulus [41].

4. Conclusions

The main focus of this study was to prepare a hydrogel of low cost, with good absorption characteristics and other significant properties (thermal and viscoelastic) for potential application in agriculture. Based on the preliminary results obtained, the novel whey/cellulose hydrogel cross-linked by CA displayed an ability to absorb large amounts of water, while also exhibiting appropriate thermal stability and viscoelasticity. Regarding the swelling ratio of hydrogel, the sample denoted as H5 (cross-linked by 5% of CA) exceeded 1600% SR, making it comparable with commercially available cellulose-based superabsorbent materials and synthetic (polyacrylate) SAP hydrogels. Moreover, combination of CMCNa/HEC polymers increased the absorption properties of the resultant hydrogel. Data on swelling kinetics revealed its promising capacity for water uptake, sustainable after 5 drying-swelling cycles. Characterizing the hydrogel brought to light the pH-dependent swelling behaviour of the novel hydrogel. All the hydrogel samples demonstrated a significantly better swelling ratio at higher pH levels of the immersion media utilized, although lower SR values was gauged in acidic media. It was also noticed that when the concentration of CA was increased, the swelling ratio of the hydrogel dropped. Hence, appropriate levels of such concentration for cross-linking and the conditions under which swelling takes place are prerequisites for preparing a hydrogel with superior swelling properties. In summary, the novel whey/cellulose-based hydrogel described herein would seem to be a suitable material for agricultural use, especially for water retention in arid areas, and may ultimately replace synthetic acrylic-based absorbents.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that no conflict of interest exists regarding the publication of this paper.

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References

- [1] P. Calcagnile, T. Sibillano, C. Giannini, A. Sannino, and C. Demitri, "Biodegradable poly(lactic acid)/cellulose-based superabsorbent hydrogel composite material as water and fertilizer reservoir in agricultural applications," *Journal of Applied Polymer Science*, vol. 136, no. 21, pp. 1–9, 2019.
- [2] E. M. Ahmed, "Hydrogel: preparation, characterization, and applications: a review," *Journal of Advanced Research*, vol. 6, no. 2, pp. 105–121, 2015.
- [3] J. Zhang, A. Li, and A. Wang, "Study on superabsorbent composite. VI. Preparation, characterization and swelling behaviors of starch phosphate-graft-acrylamide/attapulgite superabsorbent composite," *Carbohydrate Polymers*, vol. 65, no. 2, pp. 150–158, 2006.
- [4] F. Nnadi and C. Brave, "Environmentally friendly superabsorbent polymers for water conservation in agricultural lands," *Journal of Soil Science and Environmental Management*, vol. 2, no. 7, pp. 206–211, 2011.
- [5] B. Narjary, P. Aggarwal, S. Kumar, and M. D. Meena, "Significance of hydrogel and its application in agriculture," *Indian Farming*, vol. 62, no. 10, pp. 15–17, 2013.
- [6] W. Abobatta, "Impact of hydrogel polymer in agricultural sector," *Advances in Agriculture and Environmental Science: Open Access*, vol. 1, no. 2, pp. 59–64, 2018.
- [7] Y. Liu, J. J. Xie, M. F. Zhu, and X. Y. Zhang, "A study of the synthesis and properties of AM/AMPS copolymer as superabsorbent," *Macromolecular Materials and Engineering*, vol. 289, no. 12, pp. 1074–1078, 2004.
- [8] K. K. Mali, S. C. Dhawale, R. J. Dias, N. S. Dhane, and V. S. Ghorpade, "Citric acid crosslinked carboxymethyl cellulose-based composite hydrogel films for drug delivery," *Indian Journal of Pharmaceutical Sciences*, vol. 80, no. 4, pp. 657–667, 2018.
- [9] E. Isikci Koca, G. Bozdag, G. Cayli, D. Kazan, and P. Cakir Hatir, "Thermoresponsive hydrogels based on renewable resources," *Journal of Applied Polymer Science*, vol. 137, no. 28, article 48861, 2020.
- [10] D. Rico-García, L. Ruiz-Rubio, L. Pérez-Alvarez, S. L. Hernández-Olmos, G. L. Guerrero-Ramírez, and J. L. Vilas-Vilela, "Lignin-Based hydrogels: synthesis and applications," *Polymers*, vol. 12, no. 1, p. 81, 2020.
- [11] P. M. Godwin, Y. Pan, H. Xiao, and M. T. Afzal, "Progress in preparation and application of modified biochar for improving heavy metal ion removal from wastewater," *Journal of Bioresources and Bioproducts*, vol. 4, no. 1, pp. 31–42, 2019.
- [12] C. M. Ewulonu, X. Liu, M. Wu, and H. Yong, "Lignin-containing cellulose nanomaterials: a promising new nanomaterial for numerous applications," *Journal of Bioresources and Bioproducts*, vol. 4, no. 1, pp. 3–10, 2019.

- [13] F. Wang, Y. Pan, P. Cai, T. Guo, and H. Xiao, "Single and binary adsorption of heavy metal ions from aqueous solutions using sugarcane cellulose-based adsorbent," *Bioresource Technology*, vol. 241, pp. 482–490, 2017.
- [14] G. Cannazza, A. Cataldo, E. de Benedetto, C. Demitri, M. Madaghiele, and A. Sannino, "Experimental assessment of the use of a novel superabsorbent polymer (SAP) for the optimization of Water consumption in agricultural irrigation process," *Water*, vol. 6, no. 7, pp. 2056–2069, 2014.
- [15] C. Demitri, M. Madaghiele, M. Grazia Raucci, A. Sannino, and L. Ambrosio, "Investigating the structure-related properties of cellulose-based superabsorbent hydrogels," in *Hydrogels-Smart Materials for Biomedical Applications*, IntechOpen, 2019.
- [16] C. Demitri, R. del Sole, F. Scalerà et al., "Novel superabsorbent cellulose-based hydrogels crosslinked with citric acid," *Journal of Applied Polymer Science*, vol. 110, no. 4, pp. 2453–2460, 2008.
- [17] A. Sannino, C. Demitri, and M. Madaghiele, "Biodegradable cellulose-based hydrogels: design and applications," *Materials*, vol. 2, no. 2, pp. 353–373, 2009.
- [18] S. Gorgieva and V. Kokol, "Synthesis and application of new temperature-responsive hydrogels based on carboxymethyl and hydroxyethyl cellulose derivatives for the functional finishing of cotton knitwear," *Carbohydrate Polymers*, vol. 85, no. 3, pp. 664–673, 2011.
- [19] J. M. B. Domingos, S. Puccio, G. A. Martinez et al., "Cheese whey integrated valorisation: production, concentration and exploitation of carboxylic acids for the production of polyhydroxyalkanoates by a fed-batch culture," *Chemical Engineering Journal*, vol. 336, pp. 47–53, 2018.
- [20] A. Abaee, M. Mohammadian, and S. M. Jafari, "Whey and soy protein-based hydrogels and nano-hydrogels as bioactive delivery systems," *Trends in Food Science & Technology*, vol. 70, pp. 69–81, 2017.
- [21] A. K. Alsaed, R. Ahmad, H. Aldoomy et al., "Characterization, concentration and utilization of sweet and acid whey," *Pakistan Journal of Nutrition*, vol. 12, no. 2, pp. 172–177, 2013.
- [22] M. Henriques, D. Gomes, and C. Pereira, "Liquid whey protein concentrates produced by ultrafiltration as primary raw materials for thermal Dairy Gels," *Food Technology and Biotechnology*, vol. 55, no. 4, pp. 454–463, 2017.
- [23] C. Demitri, F. Scalerà, M. Madaghiele, A. Sannino, and A. Maffezzoli, "Potential of cellulose-based superabsorbent hydrogels as water reservoir in agriculture," *International Journal of Polymer Science*, vol. 2013, Article ID 435073, 6 pages, 2013.
- [24] A. Sannino, A. Esposito, A. De Rosa, A. Cozzolino, L. Ambrosio, and L. Nicolais, "Biomedical application of a superabsorbent hydrogel for body water elimination in the treatment of edemas," *Journal of Biomedical Materials Research*, vol. 67A, no. 3, pp. 1016–1024, 2003.
- [25] A. M. Abdallah, "The effect of hydrogel particle size on water retention properties and availability under water stress," *International Soil and Water Conservation Research*, vol. 7, no. 3, pp. 275–285, 2019.
- [26] Y. J. Zhou, P. Luner, and P. Caluwe, "Mechanism of crosslinking of papers with polyfunctional carboxylic acids," *Journal of Applied Polymer Science*, vol. 58, no. 9, pp. 1523–1534, 1995.
- [27] Z. Sabzalian, M. N. Alam, and T. G. M. van de Ven, "Hydrophobization and characterization of internally crosslink-reinforced cellulose fibers," *Cellulose*, vol. 21, no. 3, pp. 1381–1393, 2014.
- [28] M. N. Alam, M. S. Islam, and L. P. Christopher, "Sustainable production of cellulose-based hydrogels with superb absorbing potential in physiological saline," *ACS Omega*, vol. 4, no. 5, pp. 9419–9426, 2019.
- [29] G. Kowalski, K. Kijowska, M. Witczak, L. Kuterasiński, and M. Lukasiewicz, "Synthesis and effect of structure on swelling properties of hydrogels based on high methylated pectin and acrylic polymers," *Polymers*, vol. 11, no. 1, pp. 114–116, 2019.
- [30] S. M. Mammadova, S. Z. Tapdigov, S. F. Humbatova et al., "Synthesis, structure and swelling properties of hydrogels based on polyacrylic acid," *Asian Journal of Chemistry*, vol. 29, no. 3, pp. 576–580, 2017.
- [31] T. Yoshimura, K. Matsuo, and R. Fujioka, "Novel biodegradable superabsorbent hydrogels derived from cotton cellulose and succinic anhydride: synthesis and characterization," *Journal of Applied Polymer Science*, vol. 99, no. 6, pp. 3251–3256, 2006.
- [32] Y. Seki, A. Altinisik, B. Demircioğlu, and C. Tetik, "Carboxymethylcellulose (CMC)-hydroxyethylcellulose (HEC) based hydrogels: synthesis and characterization," *Cellulose*, vol. 21, no. 3, pp. 1689–1698, 2014.
- [33] H. Zand-Rajabi and A. Madadlou, "Citric acid cross-linking of heat-set whey protein hydrogel influences its textural attributes and caffeine uptake and release behaviour," *International Dairy Journal*, vol. 61, pp. 142–147, 2016.
- [34] B. Ozel, S. Cikrikci, O. Aydin, and M. H. Oztop, "Polysaccharide blended whey protein isolate-(WPI) hydrogels: a physicochemical and controlled release study," *Food Hydrocolloids*, vol. 71, pp. 35–46, 2017.
- [35] S. Gunasekaran, L. Xiao, and M. M. Ould Eleya, "Whey protein concentrate hydrogels as bioactive carriers," *Journal of Applied Polymer Science*, vol. 99, no. 5, pp. 2470–2476, 2006.
- [36] S. M. H. Bukhari, S. Khan, M. Rehanullah, and N. M. Ranjha, "Synthesis and characterization of chemically cross-linked acrylic acid/gelatin hydrogels: effect of pH and composition on swelling and drug release," *International Journal of Polymer Science*, vol. 2015, Article ID 187961, 15 pages, 2015.
- [37] M. R. Guilherme, F. A. Aouada, A. R. Fajardo et al., "Superabsorbent hydrogels based on polysaccharides for application in agriculture as soil conditioner and nutrient carrier: a review," *European Polymer Journal*, vol. 72, pp. 365–385, 2015.
- [38] M. J. Tavera-Quiroz, J. Ferial Díaz, and A. Pinotti, "Characterization of methylcellulose based hydrogels by using citric acid as a crosslinking agent," *International Journal of Applied Engineering Research*, vol. 13, no. 17, pp. 13302–13307, 2018.
- [39] A. Vijayan, S. Muralidharan, and N. John, "Hydrogel prepared from biomaterials specifically from carboxymethyl cellulose and its blend with starch for metal removal from water," *International Journal of Recent Engineering Research and Developmen*, vol. 3, no. 7, pp. 64–72, 2018.
- [40] M. H. Chen, L. L. Wang, J. J. Chung, Y. H. Kim, P. Atluri, and J. A. Burdick, "Methods to assess shear-thinning hydrogels for application as injectable biomaterials," *ACS Biomaterials Science & Engineering*, vol. 3, no. 12, pp. 3146–3160, 2017.
- [41] A. S. Queirós and J. A. Lopes-da-Silva, "Nonthermal gelation of whey proteins induced by organic acids," *Journal of Applied Polymer Science*, vol. 134, no. 30, pp. 1–10, 2017.