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# Preparation of chemically crosslinked chitosan hydrogels with adipic acid and EDAC carbodiimide: Tailored biomaterials for multifunctional applications

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## Abstract

Hydrogels are versatile biomaterials with significant potential in agriculture, food preservation, and biomedical applications. Hydrogels are three-dimensional networks of hydrophilic polymers that can absorb and retain large amounts of water or biological fluids. Among the polymers suitable for hydrogel synthesis, chitosan is particularly notable due to its biocompatibility, biodegradability, and antimicrobial properties. This study investigates chitosan characteristics, focusing on its various properties and how they influence its potential applications in the synthesis of chitosan hydrogels crosslinked with adipic acid using the water-soluble carbodiimide N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) to develop multifunctional films for practical applications. The raw materials were characterized using potentiometric titration, Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). The hydrogels were analyzed for morphology and crystallinity through Scanning Electron Microscopy (SEM), FTIR and XRD, comparing samples with and without the addition of the carbodiimide crosslinker. Quantitative analysis of swelling behavior was also conducted using standard buffer solutions. The results showed that crosslinking with adipic acid enhanced the crystallinity of chitosan, while the inclusion of EDAC reduced crystallinity and the degree of swelling, indicating improved crosslinking between polymer chains. These changes in structural and functional properties demonstrate the adaptability of these hydrogels for specific applications. This study underscores the potential of chitosan-based hydrogels as advanced biomaterials.

**Keywords:** Adipic acid, Chemical crosslinking, Chitosan, EDAC, Hydrogel, Biomaterials.

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**Contribution of this paper to the literature**
This study is original in its investigation of chitosan hydrogels crosslinked with adipic acid using EDAC, offering an alternative to commonly used acids. This method enhances properties of chitosan, with potential applications in agriculture and food sciences, particularly in controlled release and preservation technologies.

## 1. Introduction

In the field of biomaterials research, the development of novel hydrogels has been a focal point for several years, driven by their potential applications in extending food shelf life, advancing agricultural practices, and supporting biomedical innovations. A critical aspect of these studies lies in the selection of polymers for hydrogel fabrication. This selection must consider the intrinsic properties of the polymers, ensuring they synergistically impart the desired functional characteristics required for the specific target application [1-3].

In the focus of this development, biomaterials for use in a multifunctional application such as and agriculture and food science represent a major area of research today [1]. In these fields, the compatibility between the material and its intended application is crucial for success. Hydrogels exhibit properties that make them highly appealing, such as their ability to retain water, hydrophilicity, expandability, selective permeability, soft consistency, and low interfacial tension [4, 5].

Chitosan (CHI) is one of the most promising polymers for hydrogel applications, as it predominantly consists of D-glucosamine units derived from the N-deacetylation of chitin, a natural component of crustacean and insect exoskeletons [6, 7]. Chitosan stands out due to its remarkable properties, including biocompatibility, biodegradability, antimicrobial activity, and high adhesiveness, largely attributed to its polycationic nature [7].

Hydrogels are advanced materials characterized by crosslinked polymer networks that form three-dimensional structures [8]. The degree of crosslinking is a critical parameter, as it directly impacts the hydrogel's physicochemical and mechanical properties. Subtle variations in crosslinking density can alter key attributes such as swelling behavior, elasticity, permeability, and structural stability [9]. Therefore, precise control over the crosslinking process is essential to tailor hydrogels for specific applications, ensuring optimal performance and functionality.

Crosslinking is a crucial process in hydrogel synthesis, involving the interconnection of polymer chains through reactions with low molecular weight crosslinkers containing at least two functional groups. This process results in a more robust, three-dimensional polymer network, enhancing the structural integrity and mechanical properties of the hydrogel. Importantly, crosslinking agents employed for hydrogels designed as biomaterials must be non-toxic to ensure safety and suitability for their intended applications [10, 11].

The objective of this work is to evaluate the feasibility of synthesizing chitosan-based hydrogels chemically crosslinked with adipic acid through a reaction mediated by the water-soluble carbodiimide N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC). These hydrogels are designed to function as biomaterials with versatile and multifunctional applications.

## 2. Experimental

### 2.1. Materials

Chitosan powder (Cat. # 419419, Sigma-Aldrich Chemical, Milwaukee, Wisconsin, USA), high molecular weight, MW= 340.000g/mol, degree of deacetylation, DD= >75%, was used without further purification. Adipic acid (Vetec Química). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) (Fluka). All salts and reagents used were of analytical degree and Milli-Q water was used in all solutions (18.0 MΩ).

### 2.2. Chitosan Hydrogels Preparation

Chitosan hydrogels were synthesized by dissolving 0.5 g of chitosan in 50 mL of a 1% (w/w) adipic acid solution under stirring at 60 °C until complete dissolution. Gradually 4 mL of NaOH (0.1 M) were added with continuous stirring, followed by cooling the solution to 40 °C. To initiate the chemical crosslinking, three formulations were prepared by varying the addition of EDAC (carbodiimide): sample A (without EDAC), sample B (0.06% w/v EDAC), and sample C (0.11% w/v EDAC). Gelation was monitored during the stirring process, and the resulting mixtures were uniformly distributed onto Petri dishes. After 7 days at room temperature, the hydrogels were dried at 40 °C for 24 hours. The formed films were washed thoroughly with 5% (w/w) acetic acid, followed by rinsing with Milli-Q water to remove residual reagents. The films underwent a final drying step in an oven at 40 °C for 24 hours to yield the crosslinked chitosan hydrogels.

### 2.3. Raw Materials Characterization

The degree of deacetylation (DD) of chitosan was performed by the potentiometric titration method in pHmeter DIGIMED D-23 which 0.1 g of chitosan were dissolved in 10 ml of standard solution of 0.1 M HCl for protonation of present amino groups. The solution was then introduced in the previously calibrated pHmeter and initiated titration with standardized NaOH 0.1 M. A typical potentiometric titration curve was obtained [12].

The titration values were applied to Equation 1, where M represents the sample mass, $N_A$ and $N_B$ are the concentrations of the acid and base, respectively, and $V_A$ and $V_B$ are their corresponding volumes. The molar masses of the deacetylated and acetylated monomers were considered as 161 g/mol and 204 g/mol, respectively. The calculated degree of deacetylation (DD) was then compared with the reference value provided by the manufacturer.

$$\varphi = N_A V_A - N_B V_B / 1000$$
$$DD\% = \left(\varphi / M - \frac{161\varphi}{204} + \varphi\right) x\ 100 \quad (1)$$

The analysis of X-ray diffraction (XRD) of the chitosan and adipic acid was performed on Shimadzu XRD 7000 from the powdered material with 2θ ranging from 3.0° to 40.0° with a step 0,06°. Chitosan was evaluated based on

the monoclinic and orthorhombic structures using it for copper Kα radiation[6] the areas under the curve were determined using baseline correction and integration, identifying the crystalline fraction ($A_C$) and amorphous fraction ($A_A$). These areas were calculated using the Microcal Origin® 8.5 software. The degree of crystallinity (GC) was then estimated using Equation 2.

$$GC = A_C/(A_C + A_A) \times 100 \quad (2)$$

FTIR spectra of the samples were acquired using a Bruker ALPHA II FT-IR spectrometer, operating at a resolution of 4 $cm^{-1}$ with 32 scans accumulated for each measurement.

### *2.4. Hydrogels Characterization*

Qualitative visual observations were made taking into account the solubility, miscibility, coloring and phase segregation of the films. The average film thickness was measured with a Mitotoyo (Â±10µm) micrometer.

Morphological analysis of the films was performed in scanning electron microscopy (SEM), equipment SHIMADZU SSX-550. The samples were covered with a thin gold layer by "*sputtering*" and positioned at the the maximum distance of the target, thus preventing damage to the films.

The X-Ray Diffraction (XRD) was performed on SHIMADZU XRD 7000 equipment with 2θ ranging from 3.0 ° to 40.0 ° with step 0.06°. The degree of crystallinity of the films was calculated using the same methodology as applied to the raw materials.

The swelling degree (SD) determination, the films were carefully weighed and transferred to an Erlenmeyer flask containing 50 mL of buffer solution at pH 4 and pH 7 (Hanna Instruments, Brazil). The samples were immersed in the solutions for 120 minutes, with swelling measurements taken at regular intervals (30, 60, 90, and 120 minutes) at ambient temperature. The swelling degree was calculated based on the variation between the initial mass (M0) and the final mass (Mf), as described in Equation 3.

$$SD = Mf\ x\ M0\ /\ M0\ x\ 100\% \quad (3)$$

## 3. Results and Discussion

Figure 1 shows a typical potentiometric titration curve obtained from the chitosan solution, where the points of inflection correspond to neutralization of the excess HCl solution and neutralization of free amino groups present in the sample respectively. For better observation Figure 1 shows the values of the first derivative of chitosan's potentiometric titration curve.

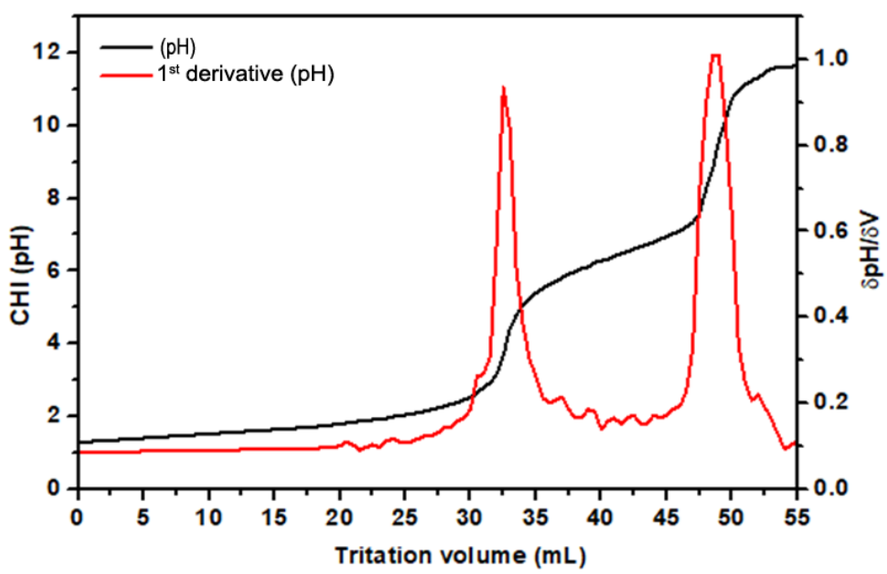


**Figure 1.** Potentiometric titration curve of CHI and first derivative curve.

Based on the observed curve and the calculations performed using Equation 1, which relies on the volume of NaOH consumed at the second inflection point, the molar quantity of -$NH_2$ groups in the sample was determined. This analysis yielded a degree of deacetylation (DD) of approximately 76% ± 6, aligning closely with the value provided by the manufacturer (>75%) for the raw material.

Figure 2 shows the diffractogram obtained from the sample of chitosan where two characteristic peaks are observed, the first of high intensity at 19.8° (d = 0.441 nm) and a lower intensity at 37.83° (d = 0.244 nm). The diffractogram highlights the crystalline phase of the sample; however, a broad baseline below the peaks indicates the presence of an amorphous phase in the feedstock. The observed angles in the diffractogram can be attributed to specific crystallographic planes (2 0 0) for the high-intensity (1 2 4) to the low intensity. From the diffractogram of chitosan, it was possible to estimate the crystallinity by 17% [13, 14].

Figure 3 shows the diffractogram obtained from the sample of adipic acid where four peaks of highest intensity are observed, the first at 12.9° (d = 0.669 nm), the second high intensity at 21.4° (d = 0.411 nm), the third at 25.7° (d = 0.345 nm) and fourth peak at 31.2° (d = 0.289 nm) After analysis and calculations it was observed that the sample is 100% crystal clear.

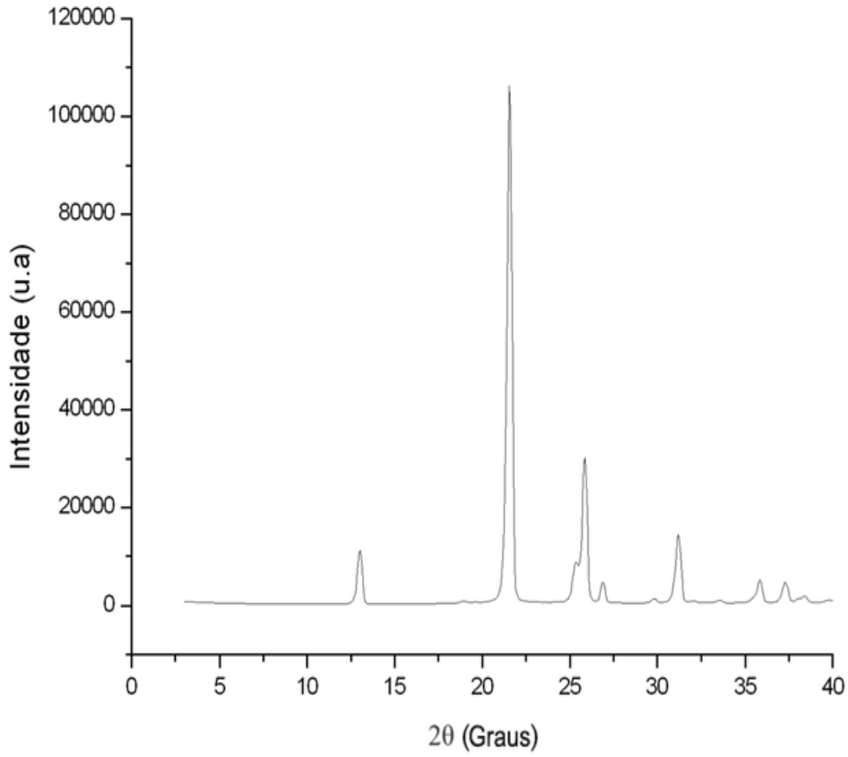


**Figure 2.** Diffractogram of raw material chitosan.

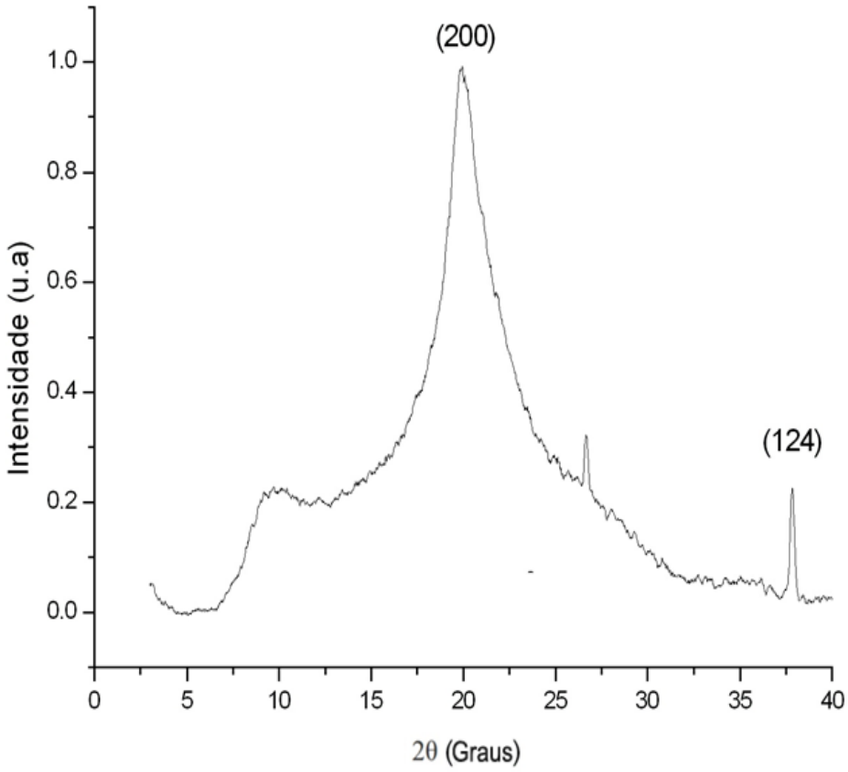


**Figure 3.** Diffractogram of the raw material adipic acid.

Figure 4 shows the FTIR spectrum of chitosan, highlighting its characteristic functional groups. Key absorption bands include the broad band around 3400 $cm^{-1}$, attributed to O-H and N-H stretching vibrations, and peaks near 2900 $cm^{-1}$ associated with C-H stretching. The amide I band, typically found around 1650 $cm^{-1}$, and the amide II band, near 1550 $cm^{-1}$, are indicative of residual acetyl groups. Additionally, the peak at approximately 1375 $cm^{-1}$ corresponds to the C-H bending of the $CH_3$ groups, while the region around 1150-1025 $cm^{-1}$ reflects the C-O-C stretching of the glycosidic bonds in the polysaccharide backbone. These spectral features confirm the molecular structure and degree of deacetylation of the chitosan sample [15, 16].

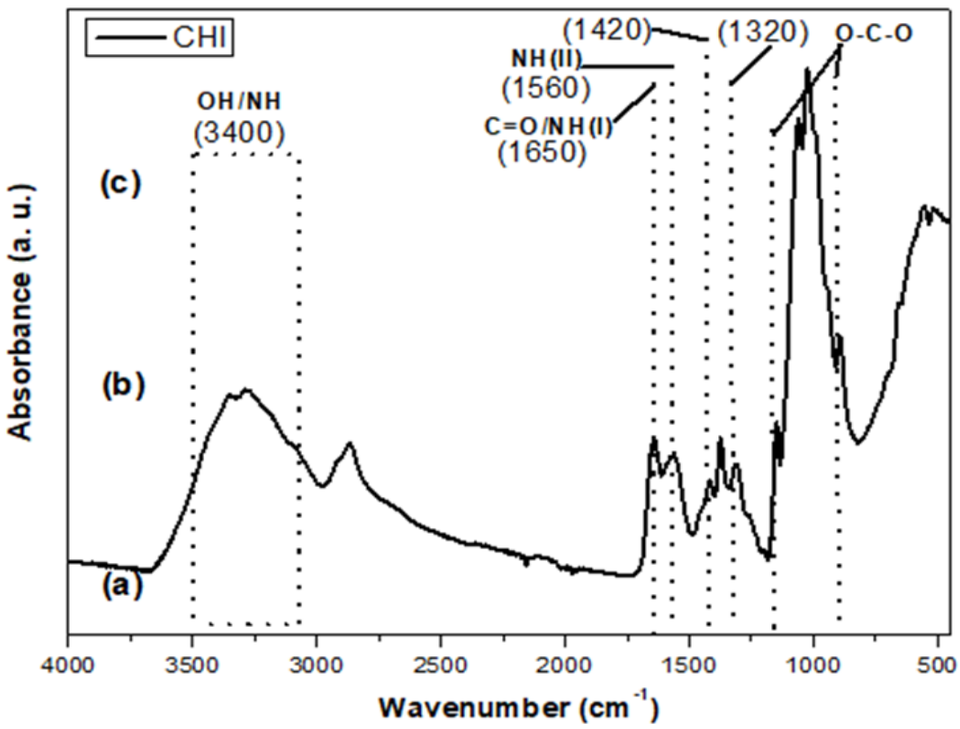


**Figure 4.** FTIR spectrum of chitosan showing its characteristic functional groups.

Figure 5 presents chitosan and adipic acid films in three conditions: without crosslinking (Film A), crosslinked with 0.06% EDAC (Film B), and crosslinked with 0.11% EDAC (Film C). All films exhibit a thickness of approximately (75 ± 25μm).

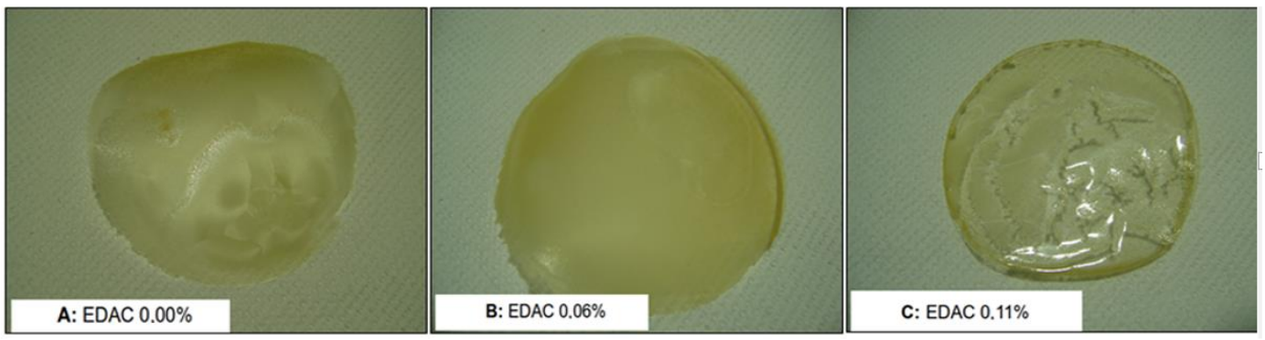


**Figure 5.** Chitosan films with adipic acid were: (A) 0.0%, (B) 0.6% and (C) 0.11% of EDAC.

A qualitative analysis of the films was performed through visual observation, and the results are summarized in Table 1. This analysis provides insights into the physical appearance, homogeneity, transparency, and surface characteristics of the films under different crosslinking conditions.

**Table 1.** Evaluation by qualitative observation of the films produced.

| Identification of hydrogels | Segregation | Opacity | Coloring | Flexibility |
|---|---|---|---|---|
| A | Segregated | Opaque | Yellowish | Flexible |
| B | Segregated | Opaque | Yellowish | Flexible |
| C | Segregated | Transparent portions | Yellowish | Flexible |

Figure 6 shows the SEM images of the films, revealing dendritic formations resulting from the crystallization process during film formation. These formations are noticeably reduced with the addition of EDAC, indicating its effect on modifying the film structure. Comparing the images of Films A, B, and C, Film C appears to exhibit a more amorphous structure, likely due to the increased crosslinking in comparison to Films A and B.

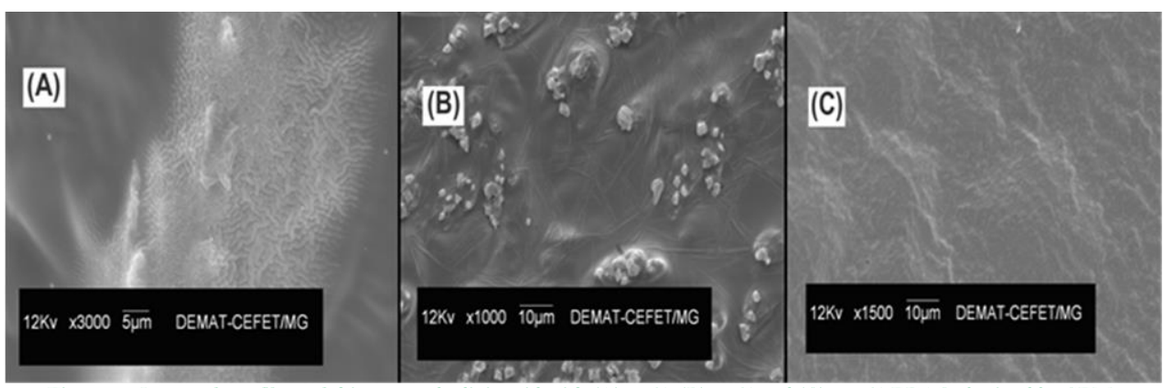


**Figure 6.** Images from films of chitosan and adipic acid with (A) 0.0%, (B) 0.6% and (C) 0.11% EDAC obtained by SEM.

In analyzing the diffractogram of the films compared to that of pure chitosan (raw material), it was observed that areas of the peaks representing the chitosan showed a relative decrease mainly after the addition of EDAC. The relative crystallinity of the films was higher than that of pure chitosan due to the high crystallinity obtained with the addition of adipic acid. After the addition of the activator (EDAC), there is a reduction in crystallinity from the diffractograms of the films B (48%) and C (45%) compared with the film A (88%) (Figure 7), suggesting that the occurrence of crosslinking reduces the mobility of the chains, preventing their crystalline organization. This result was anticipated, as the addition of EDAC promotes chemical crosslinking, which takes precedence over the ionic crosslinking naturally present in chitosan films when combined with diacids. This shift enhances the structural integrity and reduces the crystallization tendencies of the films [9, 17].

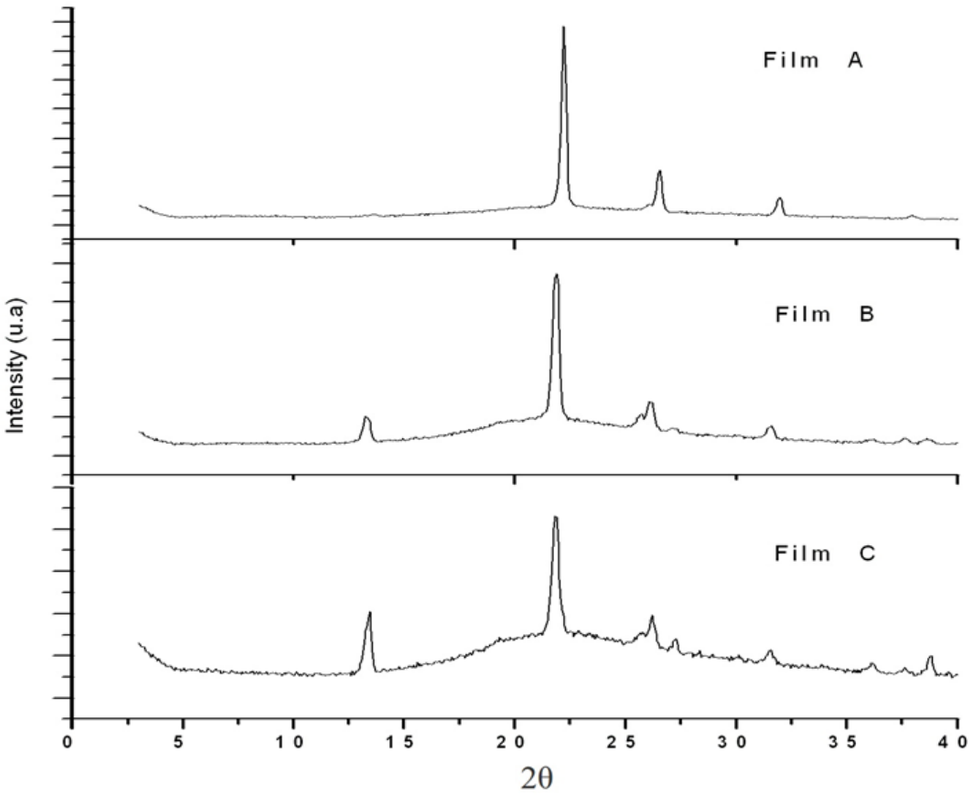


**Figure 7.** Diffractogram of the films A (EDAC 0,00%), B (EDAC 0,06%) and C (EDAC 0,11%).

Figure 8 illustrates the swelling curves of the films at pH 4, while Figure 9 shows the swelling degree of the films at pH 7. These figures provide a comparative analysis of the films' responsiveness to different pH conditions, highlighting the influence of pH on their swelling behavior.

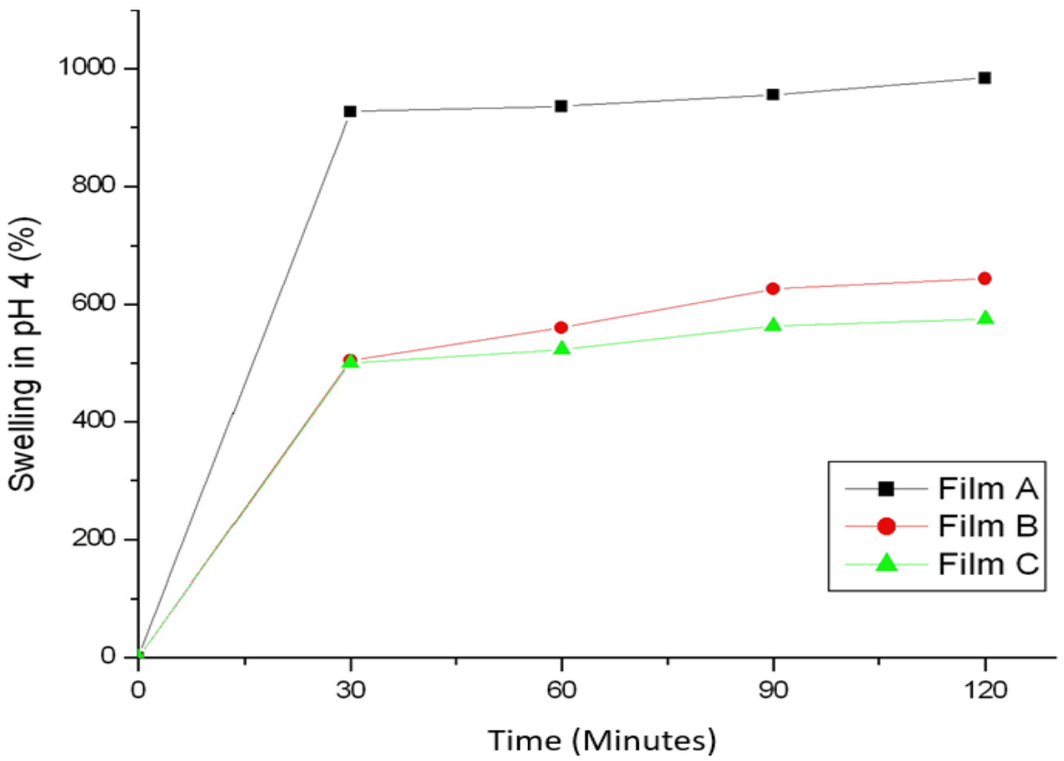


**Figure 8.** Profile of swelling of the films A (EDAC 0,00%), B (EDAC 0,06%) and C (EDAC 0,11%) at pH 4.

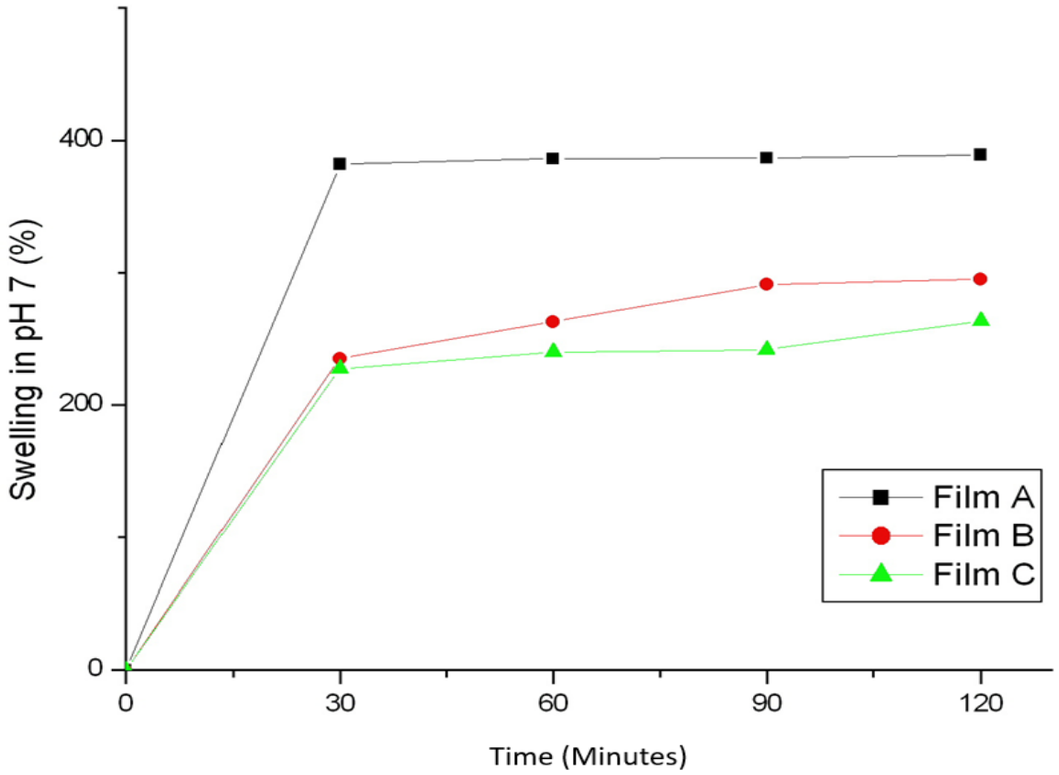


**Figure 9.** Profile of swelling of the films A (EDAC 0,00%), B (EDAC 0,06%) and C (EDAC 0,11%) at pH 7.

The swelling process occurs in three main steps: 1) Diffusion of solvent molecules into the polymeric network; 2) Relaxation of polymer chains as a result of hydration; and 3) Expansion and swelling of the polymeric network, also known as matrix swelling. However, as the degree of crosslinking increases, fewer hydrophilic groups are exposed to water, reducing their ability to diffuse through the polymer network. Consequently, this results in a lower swelling degree for Films B and C compared to Film A, which has a lower degree of crosslinking [18, 19].

## 4. Conclusion

Chitosan films were prepared using adipic acid, with or without the addition of the acid activator, a water-soluble carbodiimide. The characterization of the raw material not only confirmed the chemical composition of the material but also allowed for analysis and comparison with the films produced. The film characterization revealed that the use of adipic acid as a crosslinking activator enhances the reactivity of the films due to its functional groups, promoting covalent crosslinking over ionic crosslinking. Additionally, changes in the crystallinity pattern of the films were observed. The addition of carbodiimide resulted in an increased amorphous portion of the film, which correlated with a decrease in the degree of swelling. This reduction in swelling is associated with more efficient crosslinking within the films.

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