

Characterization of Methylcellulose Based Hydrogels by Using Citric Acid as a Crosslinking Agent

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Abstract

This research work aims at studying citric acid crosslinking capacity, evaluating structural modifications produced on hydrogels based on methylcellulose and sorbitol, along with the effect on the barrier properties of the obtained hydrogels. This additive, besides its preservative capacity in foods, can act as a crosslinking agent in some polymers matrix. Methylcellulose (A4M) solutions were prepared at 1.5% / v at 80 °C. Sorbitol analyzed concentration was 0.25%. Citric acid was added to 5 and 10 (p/pMC) concentrations as a crosslinker agent in hydrogels forming solutions. Hydrogels were obtained by casting and drying method. Moisture content, swelling capacity and films' solubility in water at different temperatures were evaluated. Hydrogels' microstructural and mechanical properties were determined through DMTA and FTIR. Water vapor permeability was determined according to the ASTM E96 standard, and oxygen permeability through an Oxtran 2/20 permeability meter by following the method described in ASTM standard. All experiments were performed on triplicate samples. ANOVA was used to analyze results by using the statistical program INFOSTAT. Formulation with better properties: low permeability to water vapor and oxygen, lower swelling capacity and greater mechanical strength was the result of combining MC with 0.25% sorbitol and 5% AC additive (w / w MC). Studies carried out on microstructural Fourier-transform infrared spectroscopy (FTIR) and barrier properties (permeability to water vapor and gases), and tensile strength tests (Dynamic Mechanical Analysis, DMA) of hydrogels obtained from cellulose derivatives (MC) confirmed that polymer's crosslinking capacity is enhanced in the presence of citric acid. Hydrogels could have applications as a controlled release agent of nutrients and / or antioxidants in food and fertilizers in soils for agriculture by using electro-stimulation techniques and / or taking advantage of their swelling capacity. They can also be used as a support for biopreservatives or as a model system for edible coatings.

Keywords: Methylcellulose; Crosslinking, Citric acid; Active film

INTRODUCTION

Cellulose derivatives such as methylcellulose and other modified natural polymers are generally used as granulation agents and in formulations for direct compression due to their binding and adhesive properties. MC, like other cellulose derivatives, is biodegradable, unlike other widely used water-soluble polymers, such as carbopol, polyvinylpyrrolidone and salts of polyacrylic acid¹ Another important application is as a film maker. Films of high transparency and strength can be obtained from MC aqueous solutions². They are water soluble and resistant to most organic solvents, fats and oils. They can be made more elastic and flexible by adding a plasticizer, such as sorbitol, glycerol or polyols^{3,4}. Like starch, methylcellulose has a large number of free hydroxyl groups, giving the resulting films, a highly hydrophilic character^{5,6}.

Water is the main biopolymer plasticizer. Addition of plasticizer to biodegradable materials generally increases their permeability and affects their mechanical properties^{7,8}. Kilburn et al.⁹ (2004) state that the effect of water plasticization on carbohydrates is generated through complex mechanisms involving both the formation of hydrogen bonds and the interruption and changes in the matrix free volume. Plasticizers (low molecular weight components) increase polymer free volume, and consequently the polymer network becomes less dense due to decrease in intermolecular forces, thus, improving films extensibility and flexibility.^{10,11}

Most effective plasticizers are those having polymer-like structures that plasticize. Polyols such as sorbitol are generally used for films from cellulose and starch^{12,13,14,15}. Sorbitol is a pharmaceutical product commonly used as an excipient to improve stability of proteins and other molecules during

freezing, drying and storage^{16,2}. Chemical modifications such as crosslinking can provide viable routes to improve mechanical properties and stability, and reduce their dissolution in water for use as controlled release agents or as edible containers^{17,18}. Polymeric matrices can be modified by chemical, physical or enzymatic cross-linking reactions^{19,20,21}. Crosslinking is an effective alternative in the properties improvement of films obtained from biopolymers. It provides greater stability and mechanical strength, which allows to get a stable matrix, with swelling capacity, good mechanical properties, thermal stability and greater resistance to dissolution in water^{22,23,24}. As mentioned above, they are useful for active agents release and as a support for cell growth^{25,26,27}.

Several authors have reported on the cross-linking capacity of some organic acids. For example, action of ferulic and tannic acid on gelatin and chitosan matrices^{28,26} and citric acid in cellulose, chitosan and starch matrices has been reported.^{29,30,18,31} Zhou et al.³² state that the effectiveness of carboxylic acids to crosslink increases as the reactive groups in the molecule increase, and under thermal treatments.

Citric acid is an organic tricarboxylic acid present in most fruits, especially lemon and oranges. It is non-toxic and has a wide use in food industry as a safe natural additive, since it is used as a preservative. This acid has the capacity to crosslink hydroxyl groups in cellulose and has been reported to occur at high temperatures (165- 175 °C)^{29,33}. Citric acid can also act as a plasticizer in certain concentrations³⁴. Although there have been several researches on matrices of methylcellulose and films plasticized with sorbitol, information about methylcellulose films with added sorbitol is scarce^{12,35,2}.

Hydrogels resulting from methylcellulose plasticized with sorbitol and with incorporation of citric acid at different concentrations were studied in this research work. Thus, this work's aims at studying the citric acid cross-linking capacity on methylcellulose hydrogels and to evaluate structural modifications and the effect on the physicochemical, mechanical and oxygen barrier properties and water vapor thereof.

MATERIALS AND METHODS

Preparation of Hydrogels Forming Solution of Methylcellulose and Citric Acid

1.5 g MC (A4M, Methocel, Dow, USA) were slowly dispersed in 50 ml distilled water at 80 °C under constant stirring for 1 h. Sorbitol (S) was added to the plasticizer agent at 0.25% concentration. From previous studies,² it was established that, with this concentration, best barrier properties and mechanical properties are obtained. At this concentration, glass transition temperature does not undergo significant changes.

Afterwards, citric acid (Biopac, CA, USA) was added at 5 and 10 (p/pMC) concentrations as a cross-linker agent in the hydrogels forming solutions. Samples without citric acid were used as control. In all cases a total 100 ml volume was made up with cold distilled water. Hydrogels were prepared by

casting and drying in an oven until reaching a constant weight and then stored at 20°C and 65% RH in a controlled room.

Hydrogels Physicochemical Characterization

Moisture Content Determination

Hydrogels moisture contents were determined by measuring their weight loss until constant weight (AOAC, 1980). Moisture results were expressed as grams of water per 100 g of dried sample (ds).

Water Barrier Properties

Hydrogels Solubility and Water Uptake

MC-based were cut in 4 cm² pieces to determine hydrogels solubility. Samples were weighed and placed into a metallic mesh. After that, they were immersed into distilled water at room temperature and kept under constant agitation. After soaking, the hydrogels' remaining pieces were dried again in an oven at 105 ± 1 °C until reaching constant weight. Solubility (%) was calculated as it was described by Rivero et al²⁴.

Water Vapor Barrier Properties

Water vapor permeability (WVP, g m⁻¹ s⁻¹ Pa⁻¹) tests were performed by using a modified ASTM method E96 as described in previous work³⁶. Each film sample was sealed over a permeation cell stored at 20 °C in a desiccator. A driving force of 1753.55 Pa, corresponding to a 75% RH gradient across the film, was used. After steady state conditions were reached, permeation cells were weighed (0.0001 g) at a 1 h interval for about 7 h. Slopes were calculated by linear regression, and reported values correspond to the average of at least three determinations.

Oxygen Barrier Properties

Hydrogels' oxygen permeability was determined using an Oxtran 2/20 permeability meter (MOCON, Modern Controls, USA), following the method described in the ASTM F1927-07 standard. Experiments were carried out at 23 °C and 65% RH. The effective area of sample' permeation was 50 cm². The samples were placed in the permeability cells and a purge was made with N₂ to eliminate traces of oxygen from the cell. Once this procedure was performed, oxygen (at atmospheric pressure) was injected into one of the compartments. The O₂ that permeates through the film is captured and measured by a coulometric sensor. The permeability was calculated by dividing the gas transmission rate by the difference of the partial pressure of oxygen through the film (1 atm) and multiplying by its thickness. It was expressed as ml O₂ μm / m² day KPa.

Fourier Transform Infrared Spectra of the Hydrogels with and without Citric Acid

The Fourier transform infrared (FT-IR) spectra of samples were recorded in an IR spectrometer (Nicolet, iS10, Thermo Scientific, Madison, USA) in the wavenumber range 4000–400 cm^{-1} . Spectral analysis of the data was performed using the software Omnic 8 (Thermo Scientific, Madison, USA).

Mechanical Properties by Dynamic Mechanic Analysis

Stress-strain tests were conducted in a dynamic-mechanical thermal equipment (DMA). Film probes with a rectangular geometry (6 mm width and 30 mm length) were tested using a 0.2 N / min ramp force constant strain rate to an 18 N static force. The test was conducted at 25 °C to obtain information about the samples behaviour at room temperature.

Reproducibility of Results

All experiments were performed on triplicate samples. (ANOVA) was used to analyse results by using the statistical program Infostat v2009 software (Córdoba, Argentina). Means comparison by Fisher LSD mean were tested, and if $P < 0.05$, the difference was considered statistically significant.

RESULTS AND DISCUSSION

Transparent and flexible hydrogels were obtained for all citric acid concentrations with the addition of citric acid in the plasticized MC hydrogels (Figure 1).

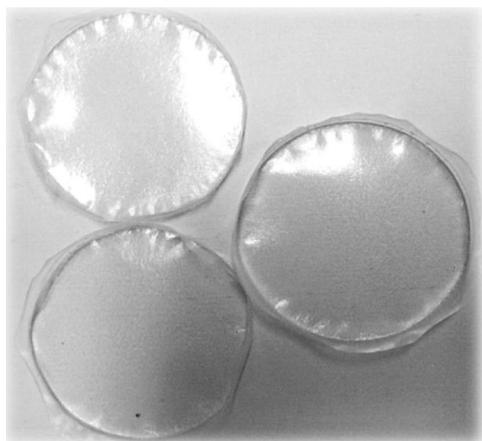


Figure 1. MC-0,25S Hydrogels with 5% Citric Acid Added (w / w MC)

Analysis of Microstructural Properties

To evaluate interaction between citric acid and methylcellulose, characterization of the resulting citric acid and hydrogels was carried out. FTIR spectrum of the citric acid powder is observed in figure 2.

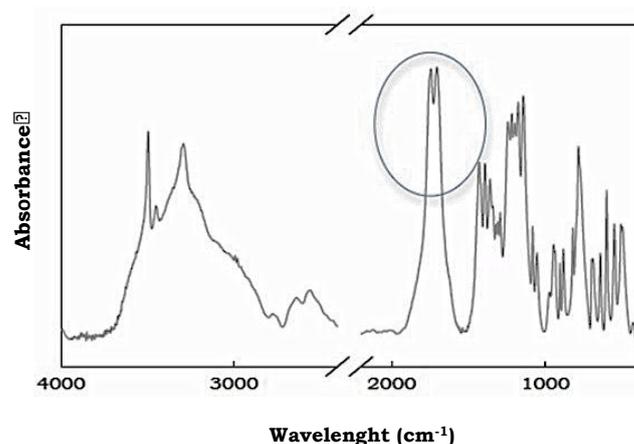


Figure 2. FTIR Spectra of Anhydrous Citric Acid Powder

Spectra of the cross-linked hydrogels were analyzed after CA characteristic bands were established. Figure 3 (a) shows the appearance of an absorption band at 1724 cm^{-1} , characteristic of carbonyls such as carboxylic acids and esters³⁸ (Figure 3b).

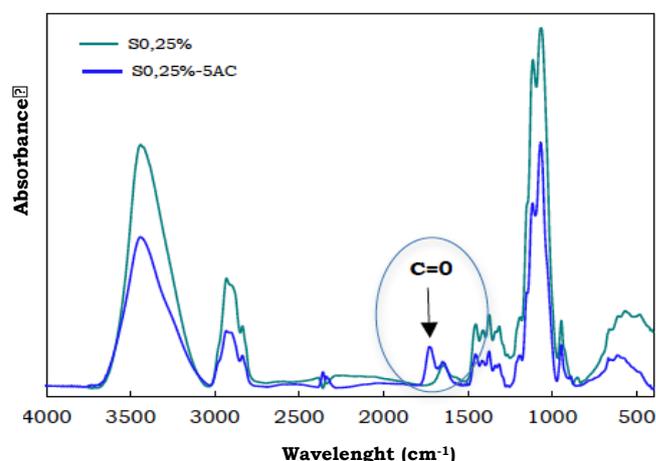
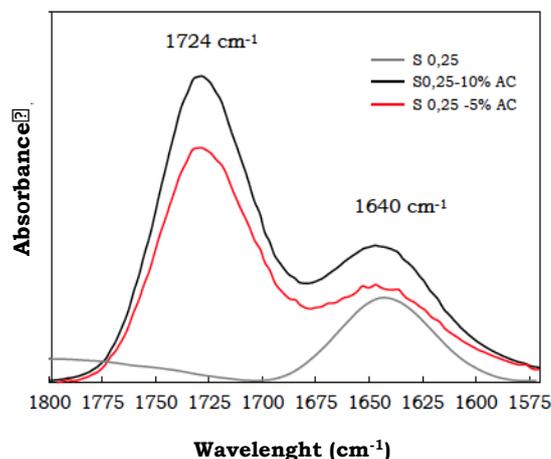


Figure 3. FTIR Spectra of a. MC Films with Added Plasticizer and 5 and 10% CA; b. Extension of the Absorption Zone between 1800-1500 cm^{-1} .



According to Olsson et al.³⁹, this band appearance shows the interaction between AC and polymer. These results were comparable with those from Rivero et al.⁴⁰ for chitosan films crosslinked with tannic acid. Other authors observed the absorption band of citric acid crosslinked with different polymer matrices^{22,24}

Mechanisms have been proposed in literature explaining the cross-linking reaction of cellulose polymers with CA. Xie et al.⁴¹ informed that when CA is heated, it is dehydrated to form a cyclic anhydride reacting with the polymer. According to Zhou et al.³² the two main stages of the reaction of polyfunctional carboxylic acids with cellulose are due to fixation of carboxylic acids through esterification with a cellulose hydroxyl group and its subsequent reaction through esterification with another cellulose hydroxyl, which produces a cross-linking between the cellulose chains.

Barrier Properties to Water Vapor and Oxygen

Table 1 shows vapor and water barrier properties, and solubility of plasticized films with 0.25% sorbitol and citric acid added.

Table 1. Barrier properties and % soluble material (100 ° C) of MC -0.25 S films with different concentrations of citric acid

Citric Acid Concentration (%)	Water Vapor Permeability (g / m s Pa) x 10 ¹¹	Permeability to O ₂ (ml O ₂ μm / m ² day Kpa)	% Soluble Material (100°C)
0	4,5(0,02) ^b	84,0 (2,1) ^c	24,5 (0,22) ^a
5	3,2(0,10) ^a	24,2 (2,4) ^a	24,4 (0,33) ^a
10	5,2(0,1) ^c	58,1 (0,1) ^b	27,6 (0,11) ^a

Different letters ^{a, b, c} in the same column indicate significant differences (P > 0.05). Values in parentheses correspond to the standard deviation.

Values from WVP and oxygen permeability (PO₂) of methylcellulose hydrogels without plasticizer addition were 8.7 x 10⁻¹¹ g / m s Pa and 128.2 ml O₂ μm / m² day KPa. According to the results, the addition of plasticizer at 0.25% concentration affected barrier properties of hydrogels in comparison with values for hydrogels without plasticizer. A decrease in PVA values was also observed for the studied CA concentrations. Incorporation of 5% citric acid (AC) to MC hydrogels plasticized with 0.25% sorbitol caused a significant decrease (P < 0.05) of PVA and PO₂ (P < 0.05). Significant differences in solubility were observed when AC content was increased to 10% (w / w MC) in the hydrogels. Rivero et al.²⁴ reported similar values of PO₂ for chitosan hydrogels crosslinked with tannic acid. According to Ou et al.⁴², cross-linking reactions restrict movement of the molecules allowing the formation of a more compact structure, which would prevent transfer molecules transfer.

Hydrogels with added AC showed a lower swelling capacity with respect to hydrogels without citric acid as observed in figure 4.

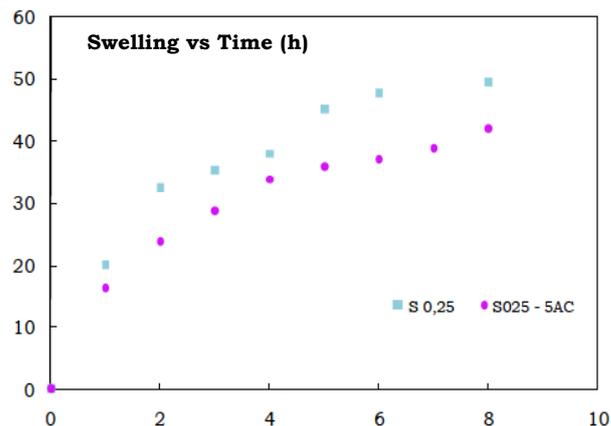


Figure 4. Swelling Capacity of MC Films with Sorbitol with 5% AC Addition.

Formation of a more compact structure after crosslinking prevents swelling of the polymer also leading to a decrease in water vapor permeability³¹. Citric acid was found to be a crosslinking agent for MC hydrogels when used in low concentrations (5% w / w). The crosslinking decreased water vapor permeability and swelling, and allowed to obtain good gas barrier properties. The formulation of MC 1.5% - 0.25% S and 5% AC (w / w MC) would allow to obtain a less affinity coating for water and oxygen, useful when required to cover foods of low humidity and preserve nutrients susceptible to oxidation.

CONCLUSION

By adding 5% CA to methylcellulose hydrogels plasticized with 0.25% sorbitol, it was possible to improve the barrier properties and decrease their affinity for water. It was possible to establish relationships between the physical properties and the content of sorbitol and citric acid of the methylcellulose hydrogels. This would allow to select optimal conditions to develop hydrogels and coatings according to specific process and storage requirements. Sorbitol would act as a protective agent due to its ability to form hydrogen bonds. This stability is crucial for the applications of hydrogels as controlled release agents or in the food industry.

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