Effect of pH on Swelling Profile of Glutaraldehyde Crosslinked Adenanthera pavonina L. Galactomannan

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Abstract. Galactomannans are neutral natural polymers isolated from legume endosperm and with wide use in the biomedical field. As a consequence, there is a need to investigate materials for their properties in order to better understand and apply them. In this study, we analyzed the influence of pH medium on the crosslinking process of galactomannan and glutaraldehyde, changing the pH medium from 3 to 7, using a strong base. The Fourier Transform Infrared Spectrum (FTIR) and the swelling profiles of the glutaraldehyde crosslinked galactomannan were evaluated. We have found that the glutaraldehyde-galactomannan crosslinking process is highly sensitive to the variation of pH medium. According to our results, best swelling capacity was achieved for samples prepared at pH=5, suggesting that this pH medium conditions is suitable for possible production of hydrogels prepared from glutaraldehyde-galactomannan.

1 Introduction
Polymers are macromolecules with physical properties that depend on factors such as chemical composition, mechanical configuration and length of the polymer chain [1]. Polysaccharides are polymers joined by glycosidic bonds in the \( \alpha \) or \( \beta \) forms. They are rich in OH groups, able to form intra or intermolecular hydrogen bridges, which favors the control of solubility [2,3].

Galactomannans are polysaccharides composed of (Figure 1) D-mannose and D-galactose [4]. They present a linear skeleton consisting of D-mannopyranose \( \beta \) - (1 \( \rightarrow \) 4). According to Dea and Morrison...
[5] there is substitution of D - mannopyranose (C - 6) hydroxyl for D - galactopyranose α - (1 → 6) in varying degrees of substitution. What differs in galactomannans is the mannose / galactose ratio as well as the species-dependent distribution throughout the skeleton of mannopyranose.

Figure 1. Galactomannan crosslinking with Glutaraldehyde.

In polymers, the crosslinking process increases mechanical and chemical resistance as well as decreases deformation. In the production of hydrogels, the crosslinking agents must have molecules with low molar mass and reactive functional groups to allow intercrossing between polymer chains [6]. In this study, *Adenanthera pavonina* L. galactomannan was crosslinked with glutaraldehyde (0.3 mol/L) using different pH medium, ranging from 3 to 7, in order to study its influence on the crosslinking process. Fourier transform infrared spectroscopy was used to identify existing functional groups and changes between different samples according to the pH variation of the preparation medium. Also the determination of the swelling degree was performed for all the samples, considering the importance of this characteristic for future applications of this biomaterials as hydrogels.

2 Methodology

2.1 Materials

*Adenanthera pavonina* L. seeds were collected in Imperatriz, Maranhão, in January 2019. They were harvested, selected, washed and kept in a cool place until use. In addition, for the crosslinking of galactomannan (Gal), glutaraldehyde (GA) chemical reagent was used.

2.2 Methods

2.2.1 Galactomannan extraction from *Adenanthera pavonina* L. seeds. In order to obtain the Galactomannan, the process started with the collection of the seeds of *Adenanthera pavonina* L., followed by the selection and cleaning of the seeds. Then they will pass through a heating and swelling stage. Finally, the seeds were manually separated from the endosperm and lyophilized.

2.2.2 Galactomannan crosslinking. A solution of 0.3 mol/L of Glutaraldehyde was prepared and its pH was adjusted with acetic acid. The solution was added to the fine powder of galactomannan and stirred for 24 hours. After stirring, the solution was placed on a petri dish for formatting. Three different galactomannan films were obtained and identified as G2G03P3, G2G03P5 and G2G03P7, according to the respective pH medium (3, 5 and 7).

2.2.3 Determination of the swelling degree of galactomannan. The samples’ swelling was obtained dipping the samples G2G03P3; G2G03P5 and G2G03P7 in 50 mL of distilled water. The immersion time periods were 20, 40, 60 and 80 minutes. After this process excess water was removed and the sample masses were weighted. Equation 1 was used to determine the swelling degree.

\[
GI = \left(\frac{(M - m)}{m}\right) \times 100
\]

Where: GI = swelling degree; M = swollen mass and m = initial mass.
2.2.4 Infrared characterization with fourier transform. FTIR spectrum was obtained from the Bruker Fourier transform spectrometer, model Vertex 70V. Potassium Bromide (KBr) pellet technique was used and each measure was performed taking 64 scans.

3 Results and discussion

3.1 Infrared Fourier Transform as a function of pH
The FTIR spectrum allows the identification of chemical groups. The transmittance data of galactomannan in the infrared region is shown in Figure 2. The band between 810-820 cm⁻¹ refers to the α-D-galactose linking unit, the band between 870-880 cm⁻¹ the β-D-mannose linkage unit [7,8], while the band between 950 cm⁻¹ - 970 cm⁻¹ was attributed to the axial deformation (C – OH) of C-4 [9]. At 1027 cm⁻¹, the band may be related to the vibrational torsion of the CH₂. Already the band at 1160 cm⁻¹ can be attributed to the module connection vibrational angular voltage δ(C―O) due to pyranose ring [9,10]. In addition, the region between 1350 cm⁻¹ and 1450 cm⁻¹, attributed to symmetrical deformations of groups CH₂ e COH [7,8]. While the wavelength at 1640 cm⁻¹ presents bands on account of the vibration of (O-H) of adsorb and stretch water molecules (C-O) of the carboxylate group (-COO-) [11]. The band centered at 1740 cm⁻¹ is associated with carbonyl C=O group. Also between 2930 cm⁻¹ and 2940 cm⁻¹ band can be referred to stretch C-H of the CH₂ grouping and the 3420 cm⁻¹ to the vibrational stretching of the group O-H [7].

![Figure 2. FTIR spectra of reticulated Galactomannan in glutaraldehyde 0.3 mol/L, in pH 3, pH 5 e pH 7.](image)

3.2 Swelling degree as a function of pH
Figure 3 shows the swelling profile of glutaraldehyde crosslinked Adenanthera pavonina L. galactomannan at pH 3, 5 and 7; It is observed that the sample G2G03P5 presented a higher swelling degree compared to G2G03P3, for a dipping period of 20 min. It is also noted that lower pH (higher H⁺ concentration) medium correspond to a decrease of the swelling degree, which should be caused by protonation of carboxylic groups in the polysaccharide structure, leading to more interactions between carboxylic and hydroxyl groups, forming more hydrogen bonds. This effect explains the decrease of free volume accessible to water molecules, lowering water penetration [12–15]. Meanwhile, the sample G2G03P7 has no swelling capacity at all since it solubilized. In addition, crosslinking hinders the mobility of the polymer chain, decreasing solubility of the polysaccharide[16]. The reticulating agent (glutaraldehyde) favors an increase of the crosslinking degree, affecting hydrogels structure. This phenomena, directly related with an higher degree of ramifications of the polymer and folding of polymer chains allied to eventual reticulation points [17], greatly affects water absorption capacity.
4 Conclusion
This research analyzed the influence of pH medium on the crosslinking process of galactomannan and glutaraldehyde. Three different pH preparation medium were used (pH=3, 5 and 7). The FTIR response of the hydrogels that resulted from crosslinking process revealed that the bands of the OH and C=O functional groups presented an increasing intensity associated to the higher pH medium. Meanwhile, our results point out that to a higher pH medium, during crosslinking, corresponds a higher swelling degree, suggesting a high sensitivity of this parameter to pH variation. Finally, considering its simplicity, this preparation method, can be easily scalable and be applied in the production of hydrogels for commercial purposes.

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