A STUDY OF SODIUM ALGINATE AND CALCIUM CHLORIDE INTERACTION THROUGH FILMS FOR INTERVERTEBRAL DISC REGENERATION USES

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ABSTRACT

The injured intervertebral disc (IVD) requires some measures in order to promote its regeneration. The sodium alginate in conjunction with CaCl₂ forms a net, potentiating its mechanical properties so it may be an alternative for IVD treatment. In this work, the viability of films of sodium alginate crosslinked with CaCl₂ and submitted to variations in their solutions’ preparations is verified, comparing the effects of the addition of CaCl₂ through their immersions, before and after drying the films. The films had their physicochemical properties analyzed by FTIR, DSC and XRD. The results indicated that films with a greater proportion of CaCl₂ were more stable in the DSC analysis when compared to films with smaller proportions of CaCl₂. These results indicate alginate’s modulation capacity which may be useful for IVD regeneration.

Key words: sodium alginate, calcium chloride, intervertebral disc, films.

INTRODUCTION

The regeneration of the intervertebral disc (IVD) is a challenging pathology which, due to the inefficiency of current treatments, demands the development of new approaches and therapeutical techniques(1). Among the most utilized materials for intervertebral discs regeneration, polymers are the most used ones. Alginate (Alg) is a natural polymer, soluble in water and composed of the repetition of two different units, (1.4)-α-L-gulunorate or unit G and (1.4)-β-D-mannuronate or unit M(2), Fig. 1. The alginate is extracted from algae and is commonly used in pharmaceutical and food industry(3). It is considered biocompatible and biodegradable and has the capacity of forming porous gel matrixes with mucoadhesive properties(4,5). An important property of alginate is its ability to interact and form gel when in contact with divalent cations, such as Ba²⁺, Ca²⁺ and Sr²⁺. The gel formation is a result of the crosslinking which occur with divalent cations, when they become lodged among the polymer chains, forming the net structure (Fig. 2).
The quantity of Ca$^{2+}$ ions present in the system interferes in the stability of these polymeric nets, forming permanent or temporary inter-chain associations. There are studies showing that the chemical structure and the size of the alginate molecule, as well as the gel formation kinetic connected to the type of ion used are determining factors on its properties, such as swellability, porosity, biodegradability, biocompatibility, gel resistance and its immunological characteristics$^7$. 

Once these materials demonstrate importance in aiding recovery from IVD injures, this work goal was to analyze and characterize the interaction between alginate and calcium chloride, as well as their physicochemical properties and biocompatibility.
MATERIALS AND METHODS

Sodium alginate powder from Sigma-Aldrich® (molar weight (MW) = 100.000 g/mol, with approximately 61% of mannuronic acid and 39% of guluronic acid, with a M/G relation of 1.56), dihydrated calcium chloride (CaCl\(_2\) from Vetec, PM 147.01) and acetic acid at 1% (CH\(_3\)COOH - Cat. #49199, Sigma-Aldrich®) were the reagents used in this work. Salts and reagents in analytical grade were used in all the solutions as well as Milli-Q water with minimal resistivity (18. 0MΩ.cm) at 25°C.

Preparation of the solution containing sodium alginate and calcium chloride (CaCl\(_2\)). The sodium alginate solution was prepared from 1% (wt/v) and kept for 24 hours under agitation of 100rpm. Later, still under agitation an aqueous solution of calcium chloride at 2% was added drop by drop. After, the system was kept under agitation for more 30 minutes. The solution was degassed under vacuum at a pressure of 350mmHg for 120 minutes. After 24 hours the solution was poured into polystyrene Petri dishes of 8.5 cm diameter and dried at 50ºC in oven boards for 24 hours. The obtained films were immersed in a calcium chloride solution at 2% (wt/v) for 1 hour and dried in room temperature for 48 hours.

Preparation of films containing sodium alginate and calcium chloride (CaCl\(_2\)). The sodium alginate and CaCl\(_2\) films were prepared as described in the previous item. Next, four mixtures were prepared as described below:
- Film 1: 100ml of sodium alginate at 1% solution (alginate control film – AC).
- Film 2: 100ml of Alg at 1% solution + 10ml of CaCl\(_2\) at 2% solution (alginate film containing CaCl\(_2\) only in the solution – ACS).
- Film 3: 100ml of Alg at 1% solution + 10ml of CaCl\(_2\) at 2% solution (alginate film containing CaCl\(_2\) at 2% both in the solution and in the immersion – ACSI).
- Film 4: 100ml of Alg at 1% solution (alginate film containing CaCl\(_2\) at 2% only in the immersion – ACI).

Characterization of the sodium alginate and calcium chloride films.
- Thermal analysis of the films. The different films were submitted to thermal analysis by differential scanning calorimetry (DSC). In order to obtain the DSC curve, samples from different films were placed in an aluminum crucible, at a 10ºC/min\(^{-1}\) heating rate, starting at 30 until 500ºC in a N\(_2\) dynamic atmosphere at a flow rate of 90mL.min\(^{-1}\)\(^{(9)}\).
- Determination of the crystallinity parameters of the films. The crystallinity parameters of the films were obtained by the X-ray diffraction (XRD) in SHIMADZU XRD 7000, with 2θ ranging from 4.00 to 90.00º and step of 0.06º(10). The degree of crystallinity was calculated based on Eq. (A).

\[ GC = \frac{AP}{AR} \times 100 \]  

(A)

- Analysis of chemical groups of films. The characterization of chemical groups was carried out by infrared spectroscopy – FTIR in a SHIMADZU Corporation spectrophotometer, model IRPrestige-21 equipped with Fourier transform, FTIR-8400S and ATR accessory between 4,000-400cm⁻¹.

RESULTS AND DISCUSSION

Differential scanning calorimetry – DSC. The DSC curve of the obtained films is presented in Fig. 3, which showed endothermic peak for the AC film at 64.8ºC, for the ACS film at 78.65ºC, for the ACSI film at 73.8ºC and for the ACI film at 86.4ºC. These endothermic peaks can be associated to the dehydration process of the sample.

![DSC curves to the sodium alginate and calcium chloride films.](image)

Figure 3 – DSC curves to the sodium alginate and calcium chloride films.

(a) AC film; (b) ACS film; (c) ACSI film; (d) ACI film.

Peaks of greater intensity were observed for the ACS and ACSI films, which may be associated to the presence of CaCl₂ in their solutions. The ACI film presented higher dehydration temperature and lower heat intensity when compared to the ACS and
ACSI films. A second exothermic peak presented apex at 209.3°C for the AC films, at 201.5°C for the ACS films, at 200.2°C for the ACSI films and 207.8°C for the ACI film. This peak could be associated to the sample degradation process. A higher peak intensity is observed for the ACS and ACSI films, which may be associated to the presence of CaCl₂ in these films’ solution. The ACSI film has higher intensity when compared to the ACS film probably due to the presence of a greater proportion of CaCl₂, both in the solution and in its immersion, granting more stability to the sample. A third exothermic peak was observed for all films. This peak was attributed to the carbonization temperature of the samples. This phenomenon was visually observed after their analysis.

**Fourier transform infrared spectroscopy – FTIR.** The infrared spectrum of the sodium alginate powder (Fig. 4) showed a broad absorption band between 3,500 cm⁻¹ and 3,100 cm⁻¹ referring to the stretching of the O-H group. As the quantity of CaCl₂ increases there is an enlargement of this region, which may be observed mainly in the ACSI film which contains the higher proportion of CaCl₂. Between 2,965 cm⁻¹ and 2,913 cm⁻¹ the band related to the stretching of the C-H symmetric and asymmetric doublet is observed, which is almost nonexistent in the ACSI film, indicating a probable interaction between sodium alginate and CaCl₂.

![FTIR spectrum of sodium alginate and calcium chloride films.](image)

- (a) AC Film; (b) ACS film; (c) ACSI film; (d) ACI film.

The wavenumbers in 1,592 cm⁻¹ and in 1,394 cm⁻¹ refer to the COO⁻ asymmetric stretching, showing an intensity decrease in this region in the films which contain
CaCl$_2$; in 1,294 cm$^{-1}$ and in 1,073 cm$^{-1}$, to the C-O stretching; in 1,119 cm$^{-1}$ and in 843 cm$^{-1}$, to the C-C stretching; in 1,018 cm$^{-1}$ to the C-O-C stretching; in 965 cm$^{-1}$, to the C-O stretching; in 912 cm$^{-1}$, to the C-C-H; in 841 cm$^{-1}$, to the C-C; and in 822 cm$^{-1}$, to the C-C-O. It can be observed in the films that by increasing the CaCl$_2$ proportions there is an intensity decrease in these regions. These values are similar to those found in Nery (2014)$^{(11)}$ studies.

**X Ray Diffraction – XRD.** The degree of crystallinity of the films was estimated by using the software Microcal Origin$^\text{®}$ version 8.0. Fig. 5 presents the diffraction pattern obtained. The AC film presents a 10.7% crystallinity degree; the ACSI film, a 24%; the ACS film, a 36%; and the ACI film, a 10%. In the diffraction pattern analysis it is possible to observe that the areas where the sodium alginate peaks took place there is an increase related to calcium chloride.

![Figure 5 – Infrared spectrum of the sodium alginate and calcium chloride films.](image)

(a) AC Film; (b) ACS film; (c) ACSI film; (d) ACI film.

The peak for diffraction at around 13$^\circ$ for the sodium alginate increases in the films composed of sodium alginate and calcium chloride, it has great intensity at \( 2\theta=13^\circ \) (d= 0.657 nm) in the ACSI film and it is almost nonexistent in the ACI film. This suggests that the interaction between the sodium alginate and the calcium chloride favors the structural organization increasing the sodium alginate crystallinity. This diffraction reflex intensity proportion changes when the content of Ca$^+$ ions increases. Such results have also been observed in the studies of Fábia, Slusarezyk, Gawlowski (2005)$^{(12)}$. 
CONCLUSIONS

In the present study sodium alginate films were prepared and crosslinked with calcium chloride. The results showed that the presence of calcium in different proportions in the sodium alginate films modulate its physicochemical properties, making its structure more thermally stable and more crystalline, and may also contribute to the intervertebral disc regeneration.

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