Synthesis and characterization of calcium alginate and cellulose nanocrystal films for lead removal

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Abstract. The contamination of water with lead (Pb) is a society concern since this metal as a toxic cation. Cellulose nanocrystals (CNC) are rod-like structures with variable size, which are prepared by acid hydrolysis of cellulose natural sources, so it is a renewable, biodegradable material, with good mechanical performance. Sodium alginate (SA) is a biopolymer, biodegradable and renewable. This biopolymer can chelate bivalent cations forming gel networks. This work focuses on the combination of these two natural biopolymers to create a film of calcium alginate with CNC with the aim to remove non-essential elements on contaminated water. SA and CNC were mixed forming a dispersion, then they were left to dry and reticulated with sodium chlorate (CaCl2) solution. To determine the adsorption kinetics, the films were put in contact with Pb solution, the mixture was maintained under agitation, at constant speed and temperature, the Pb and calcium (Ca) concentrations in supernatant aliquots were determined using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES) and Flame Atomic Absorption Spectroscopy (FAAS), respectively. The results were applied on three kinetic models of adsorption: pseudo-first order; pseudo-second order and Weber-Morris. Characterizations of films were carried by Fourier Transform Infrared (FTIR); Atomic Force Microscopy (AFM); Scanning Electron Microscopy (SEM). AFM and SEM topography study showed a very flat and smooth surface for the films. The adsorption of Pb onto film increased with time contact. Maximum adsorption of Pb was 3.5 mg per gram of film. The prepared films were characterized by AFM and SEM. FTIR confirmed the structure of both polysaccharides. The Pb adsorption experimental data showed an adjusting for pseudo-second model, agreeing with published papers. Further analysis should be done to evaluate isotherms of adsorption and methods for removing the non-essential elements of the film, with the aim of a possible reuse of it.

1. Introduction

Water is an important resource since it is one of the basic needs for life. River basins are the usual resource of water for humans in cities or rural areas. Toxic and non-essential nutrients, known in the past as non-essential elements, are a common pollutant of water. Depending on the metal, its oxidation state, or if it is bonded to organic compounds, small concentrations of ng/L can compromise water supply. Unfortunately, with the raising of cities and industrialization, the presence of non-essential elements on environmental has been dramatically increasing. Pb is one of these non-essential elements, with toxic effects including damage to the nervous system; kidneys and reproductive system [1,2].
Alginate is a natural biopolymer, biodegradable and renewable, which is extracted from brown algae species as *Laminaria spp.*, *Ascophyllum nodosum* and *Macrocystis pyrifera*. The extraction is carried by neutralization of alginic acid sites with basic hydrolysis, which leads to the formation of alginate ion, which is a polyanion, since it has negative carboxylic terminals. The alginic acid structure is shown on Figure 1, in which on (a) and (b) are shown its forming blocks, and at (c), (d) and (e) show how this block can be arranged on the polymer to give different structures in the same polymer chain [3].

![Figure 1](image1.png)

**Figure 1** – Forming blocks and structures of the biopolymer alginic acid showing its carboxylic acid terminals and their different arrangements.

Physical crosslinking is the most used method of gelification of this material since is cheap and simple method, using bivalent cations that are chelated by carboxyl terminals of alginate, generally in GG and MGM structures. The interaction strength of alginate with bivalent cations varies according to the cation used. Non-essential elements, as Pb, have stronger interactions compared with and alkaline-earth metals, such as Ca [4]. As some cations have stronger interactions with alginate than others, by using calcium alginate film, due to the stronger interactions of Pb with alginate, Ca can be replaced by Pb, removing Pb ions from the effluent. However, for alginate films to exchange Ca cations to Pb cations, it is necessary to have low concentration of Ca$^{2+}$, and this fact makes films more fragile, needing another material to improve the mechanic performance of the films. Nanocellulose is a material that can be presented as cellulose nanocrystals (CNC) or cellulose nanofibrils (CNF), and it has many advantages, such as: low cost; it is a renewable and biodegradable material with chemical inertness and flexibility, and desired high mechanical performance [5]. Some works have reported nanocellulose being used as foams [6] and beads [7] for water treatment and as drug carriers [8].

Nanocellulose is prepared through acid hydrolysis of sources of cellulose, which is one of the most abundant resources of the World. Usually, when the hydrolysis is conducted with sulfuric acid, it produces more stable nanocellulose, because it forms sulfate halfesters with negative charge, which stabilize the structure [5].
This work focuses on the application of calcium-alginate films (CA films), containing CNC (CA-CNC films), for Pb removal, and the investigation of the adsorption and ion-exchanging characters of the films. The combination of alginate with CNC improved the mechanical properties of the film. However, lower concentrations of CNCs must be used, since CNC and alginate are negatively charged, then, higher concentrations of CNC could have the opposite effect, producing a weaker film.

2. Materials and methods

2.1. Materials

Cellulose NanoCrystals (CNC) were acquired from Celluforce. Sodium alginate was purchase from Sigma-Aldrich (São Paulo, Brazil). Dihydrated calcium chloride and hydrochloric acid from Synth (São Paulo, Brazil). Lead nitrate from ECIBRA (Curitiba, Brazil) and reference solution 1000 mg L⁻¹ of Pb from SpecSol (Brazil). All the solutions were prepared with Milli-Q purity grade water from (PureLab Option Q, Elga LabWater, Paris, France). All the reagents are analytical grade without further purification.

2.2. Synthesis of calcium-alginate (CA) and calcium alginate containing CNC (CA-CNC) films

Firstly, sodium alginate was solubilized in water at 70 °C for 1 h giving a 2 % w/v solution. CNC was homogenized in a Turrax homogenizer for 3 minutes for a dispersion 1 % w/v. Then, a volume of sodium alginate was mixed with a volume of CNC dispersion to result in a solution of 1 % w/v of alginate and 0.05 % w/v of CNC. Separately, it was prepared a solution of 20 mM of calcium chloride. The combined solution of sodium alginate and CNC was put in silicon molds and a vacuum of -700 mmHg was applied to remove any bubbles. The films were left to dry on 45 °C in a hot air oven for 12 h. After drying, the films were immersed in 5 mL of calcium chloride 20 mM for 1 h. The calcium concentration on the solution was measured before and after the films synthesized by Flame Atomic Absorption Spectroscopy (FAAS) (Contra 300, Jena, Germany).

2.3. CA and CA-CNC film characterization

The prepared films were characterized by Attenuated Total Reflectance (ATR) – Fourier Transform Infrared (FTIR) (Spectrum Two, PerkinElmer, Massachusetts, United States of America) from 4000 cm⁻¹ to 500 cm⁻¹, using resolution of 4 cm⁻¹. Atomic Force Microscopy (AFM) (Mx10 model, Park System, Suwon, South Korea), using intermittent contact mode with a FMR-W Silicon-SPM cantilever (Nanoworld, Neuchâtel, Switzerland). Scanning Electron Microscopy (SEM) (6010LA, JEOL, Tokyo, Japan) was used for morphological studies. Surface chemical composition were obtained by X-ray Photoelectron Spectroscopy (XPS) (K-alpha+; Thermo Fisher Scientific, Rochford, UK) equipped with hemispherical electron analyzer and monochromatic Al Kα (1486.6 eV), 10 nm of reach depth, and pass energy of 200 and 50 eV to obtain the full range and high resolution spectra, respectively.

2.4. Adsorption kinetics of Pb

CA and CA-CNC film samples of 0.2 g were immersed in 10 mL of 500 µg L⁻¹ Pb solution and the mixture was maintaining at magnetic stirring at different contact time period from 0.5 to 24 h at room temperature according to the Table 1. For comparison, this procedure was done for both films, with CNC and without CNC. A film that was not incubated in Pb solution for both groups was used as control. The assay was carried at pH 5.0 and temperature 25 °C.

| Sample name | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|-------------|---|---|---|---|---|---|---|---|
| Time (h)    | 0.5 | 1.0 | 1.5 | 2.0 | 4.0 | 6.0 | 12.0 | 24.0 |
Total dissolution of the films was carried by adding 1 mL of nitric acid, which was left for 24 h, then the films were put in a water bath at 95 °C for 4 h. Films were diluted to 50 mL to be analyzed. Pb and calcium concentrations were determined by using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES) (710 series, Varian, California, United States), using emission line of 220.353 nm for calcium and 396.847 nm for Pb. The assay was repeated 3 times.

The Pb removal kinetics were studied based on three models: pseudo-first order; pseudo-second order and Weber-Morris, also known as intraparticle diffusion model. A summary of removal kinetics and adsorption kinetics is shown in Table 2 [9,10].

| Model                          | Equation                                      |
|--------------------------------|-----------------------------------------------|
| Pseudo-1st Order              | \( \ln (q_t - q_e) = \ln (q_t) - (k_1t) \)  |
| Pseudo-2nd Order              | \( t, (q_0)^{-1} = (k_2q_e^{-2})^{-1} + (q_e)^{-1}.t \) |
| Weber-Morris (Intraparticle diffusion) | \( q_t = K_{ad} \cdot t^{1/2} + C_i \) |

\( t \) stands for time; \( q_t \) is the amount of Pb (mg) absorbed per gram of film at time \( t \); \( q_e \) is the amount of Pb (µg) absorbed per gram of film at the equilibrium; \( k_1 \) is the pseudo 1st order rate constant (h\(^{-1}\)); \( k_2 \) is the pseudo 2nd order rate constant (g . µg\(^{-1}\) . h\(^{-1}\)); \( K_{ad} \) is the constant of the diffusion of the adsorbate in step i of a Weber-Morris adsorption (µg . g\(^{-1}\) . h\(^{-1}\)); Adapted from [9]; [10].

From the plot of adsorption kinetic, the experimental \( q \) of equilibria (\( q_{e,exp} \)), as the highest concentration observed at the plateau of absorption can be determined. In addition, the angular coefficient of linearization of pseudo-second order gives the calculated \( q \) of the equilibria (\( q_{e,calc} \)). These two values were compared using two-tailed t-test, which calculated the t value by Equation 1, which \( s \) is the standard deviation of the average \( q_{e,exp} \), and if \( t < 4.30 \) or \( t > -4.30 \), then the null hypothesis is accepted with 95 % confidence [11].

\[
\text{Two-tailed t-test: } t = \frac{q_{e,exp} - q_{e,calc}}{s/\sqrt{3}} \quad (\text{Eq. 1})
\]

2.5. Swelling behavior

A known mass of CA and CA-CNC films were dried at 45 °C on hot air oven for 6 h. Then, the dried films were weighted (\( W_d \)). These films were put in 10 mL of phosphate buffer solution (pH = 7.4) for 24 h, then they were weighted again (\( W_w \)). The water absorption in % was obtained by the mathematical equation [12,13]:

\[
\text{Water absorption (%)} = \frac{(W_w - W_d) \times 100}{W_d} \quad (\text{Eq. 2})
\]

3. Results

3.1. Synthesis of CA and CA-CNC films

The synthetic method to prepare CA and CA-CNC films is relatively simple, it was performed in aqueous medium at room temperature, with the absence of residues or byproducts, in accordance with the principles of green chemistry. The drying process of the precursor solution allowed the films to get the desired shape. The reticulation time is an important parameter, since higher concentrations of calcium might decrease the ion-exchanging process. Previous tests were conducted to optimize the reticulation time, and it was found that 1 h was the optimized time.
The analysis of calcium content in the films was performed in order to estimate the total amount of Pb that might be absorbed by the films, by ion-exchanging (Ca by Pb cations). Table 3 shows the determination of calcium in the films with and without CNCs.

### Table 3 Determination of calcium content in the films in the presence and absence of CNCs, and estimated amount of Pb that can be removed by the film.

| CA film 1 % alginate: | $Q_{Ca}$ (mg . g$^{-1}$) | $Q_{mol}$ (mmol . g$^{-1}$) | $Q_{Pb}$ (mg . g$^{-1}$) |
|----------------------|--------------------------|-----------------------------|--------------------------|
| With 0.05 % CNC      | 3.58 ± 0.24              | 143.2 ± 9.6                 | 29.6 ± 2.0               |
| Without CNC          | 3.95 ± 0.15              | 158.0 ± 6.0                 | 32.7 ± 1.2               |

$Q_{Ca}$ = Mass of calcium (mg) per mass of film produced (g); $Q_{mol}$ = amount of calcium (mmol) per mass of film (g); $Q_{Pb}$ = Expected mass of Pb (mg) per mass of film (g).

3.2. Characterization of the prepared films

3.2.1. Fourier Transform Infrared (FTIR)

The FTIR spectroscopy profiles of films of CA and CA-CNC are shown in Figure 2. The spectra of CA films and CA-CNC films have no significant differences. Both films shown a wide band of -OH stretching on 3275 cm$^{-1}$; two characteristic peaks of symmetric and asymmetric stretching of -COO at 1589 cm$^{-1}$ and 1412 cm$^{-1}$, respectively; the peak of 1024 cm$^{-1}$ corresponds to the ether, and a very small peak in 2935 cm$^{-1}$ corresponds to the -CH stretching. All the peaks are correctly correlated with what is found on literature [12,14,15].

![Figure 2 - FTIR spectra of CA (red line) and CA-CNC (black line) films with attributed peaks. CA content in the films: 1 %, and CNC content in the film: 0.05%.

3.2.2. Atomic Force (AFM) and Scanning Electronic Microscopy (SEM)

AFM images of the films (CA and CA-CNCs) are shown in Figure 3, and the micrographs obtained by SEM on Figure 4. It can be observed that both films have a very smooth surface, and both micrographs obtained by of SEM and AFM are correlated. In can be observed for CA films the presence of some grooves on the film surface. The conditions of analysis were very important: on SEM, as it is a polymer, no more than 5 kV were used to avoid a film burning. On AFM, intermittent contact was used to avoid any damage to cantilever or alteration of surface.
3.2.3 X-ray Photoelectron Spectroscopy (XPS)

The full range spectra of CA film and CA-CNC film are shown in Figures 5 and 6, respectively. It can be observed peaks for carbon (C 1s); oxygen (O 1s); calcium (Ca 2p) and chlorine (Cl 2p). Table 4 present the atomic percentage of the elements for both films [16,17]. The main difference between the two spectra is the intensity of C-O and O-C-O peaks. Furthermore, the peaks of chlorine and calcium were not detected in the full range spectra of CA-CNC.
3.3. Adsorption kinetics

The plot of Q versus time; kinetics studies of pseudo-first order; pseudo-second order and Weber-Morris are shown in Figure 7; 8; 9 and 10, respectively. There was no linear correlation of data with the pseudo-first order model. The best correlations were found for pseudo-second
order, which slope and intercept can give the \( q_{e,\text{calc}} \), which is the \( Q \) of equilibria calculated using the angular coefficient, and the \( k_2 \), as shown in Table. Using two-tailed Student’s t test, it was obtained \( t = 2.843 \) and \( t = 0.323 \) for alginate and alginate-CNC films, respectively, meaning 95 \% that the differences in the values has no significant difference [11].

![Figure 7](image)

**Figure 7** – Mass of Pb adsorbed per gram of film through the time. Black dots: CA film, red dots: CA-CNC films. CA content in the films: 1 \%, and CNC content in the film: 0.05\%.

![Figure 8](image)

**Figure 8** – Pseudo-first order correlation for CA film (black line) and CA-CNC film (red line). CA content in the films: 1 \%, and CNC content in the film: 0.05\%. 
Figure 9 - Pseudo-second order correlation for CA film (black line) and CA-CNC film (red line). CA content in the films: 1%, and CNC content in the film: 0.05%.

Figure 10 – Weber-Morris kinetics of adsorption correlation for CA film (black line) and CA-CNC film (red line). CA content in the films: 1%, and CNC content in the film: 0.05%.

Table 5 – Data obtained for pseudo-second order kinetic correlation for CA and CA-CNC films.

| Films 1 % Alginate | $q_e$ _calc_ (µg . g$^{-1}$) | $q_e$ _exp_ (µg . g$^{-1}$) | $k_2$ (g . µg$^{-1}$ . h$^{-1}$) | $R^2$  |
|-------------------|-------------------------------|-----------------------------|-------------------------------|--------|
| With 0.05 % CNC   | 6.999                         | 7.844 ± 0.515               | 0.214                         | 0.9463 |
| Without CNC       | 9.331                         | 9.380 ± 0.262               | 0.072                         | 0.9802 |
It can be seen in Table 5 that with CNC, the film has a higher rate of adsorption. However, without CNC in the film, the equilibrium amount of Pb in mass adsorbed per gram of film was higher, compared with the control group. At Figure 7 it can be seen that the highest peak is reached in 2 h for CA-CNC films, while for pure alginate the highest peak is reached after 4 h. Even having adsorbed more Pb in the equilibrium, it can be pointed that without CNC the film is more fragile than with CNC. So, even having less Pb in the equilibrium, the films of alginate-CNC are still better than pure alginate films.

Comparing the maximum values obtained in the Figure 7, they are very lower than what was expected in Table 5 and this may be explained due the fact that the concentration of Pb is very low, which decrease the mobility of ions, and limits the amount of Pb that could be adsorbed, which is a observed effect in other works [18].

The pseudo-second order kinetics revealed that the initial concentration of Pb plays a significant role in the sorption. Another work found in literature studied three species of brown algae and one species of fungi, all rich in alginate, for removal of copper and Pb ions. All of them, for both cations, showed pseudo-second order kinetics of removal [2]. Another two works supported using biomass rich in alginate also supported the fact [19,20]. However, as the initial concentrations of non-essential elements used on each work are different, the k2 values cannot be compared.

The Weber-Morris, or intraparticle diffusion, also showed a high correlation constant, generally it is applied for models that has steps of diffusion before the adsorption happens, divided between three steps: the external diffusion, the internal diffusion and the equilibrium. As is shown in Figure 10, the lines generated by plotting qt versus t do not pass through the origin, when this happens it can be speculated that the rate-limiting sorption step was simultaneously governed by both film diffusion and intraparticle diffusion [10].

The pH and temperature were important, because the pH may influence the stability of cations. Xu et al and Wang et al showed that the optimal pH for adsorption was between 4 and 6 for alginate materials [21,22]. The temperature was kept at 25 °C with the aim to be a green adsorption avoiding energy.

To confirm a possible mechanism of ion exchanging, the calcium was measured in the solution after incubation with films. The results are shown in Figure 11. There is an increasing of calcium concentration in the solution with the time, proving that calcium is being exchanged for Pb during adsorption. However, it cannot be stated that the mechanism of Pb removal is purely an ion exchanging, because the peak of adsorption of Pb is at 4 h, but the peak of release of calcium is at 12 h. To sum up, it is a mix of physical and chemical adsorption. This effect is common in biomass polymers, moreover it means a great potential of reuse [18,22].
3.4. Swelling behavior

The obtained values for swelling behavior were 3939.51 ± 114.80 % and 2808.52 ± 147.74 % for films of CA and CA-CNC, respectively. The dried films absorb a great amount of water, the CA-CNC less, probably due to the presence of CNC. The 1 % alginate film was more fragile after the essay. Although, this result shows that both films have great interactions with the effluent that would guarantee a better adsorption of the non-essential elements.

4. Conclusion

In this work, a new methodology of combination of alginate and CNC as films was proposed with the aim to remove Pb from effluents. The comparison between the control film (without CNC) and the CA-CNC films showed that Pb is removed, so the aim has been achieved. With higher mechanical performance and fast Pb adsorption, the films with CNC proved to be better, even the film without CNC in the equilibrium having higher concentration of Pb. The films without CNC are more fragile, so as the time passes, in the magnetic stirring, it was possible to observe that some films of pure alginate were ripped apart. When this happens, more area of the films is exposed to adsorb the Pb, explaining why the adsorption is faster. Nevertheless, CA-CNC films are strong enough to resist stirring conditions of removal assay, being easily recovered. Furthermore, their rate of adsorption is close, despite being smaller, of what was found for films without CNC. The kinetics of the adsorption process were suggested to follow the pseudo-second-order model and controlled by diffusion and intraparticle diffusion.

Therefore, CA-CNC combination has a great potential as Pb removal. New tests should be performed in order to evaluate how the adsorption behaves in other pH conditions just for confirmation of what is found in literature; and initial concentrations of the cation, which is also important not just to study the behavior of film in other conditions but also for a isotherm study which should stand if it is purely a chemical or a physical adsorption. Also it must be testes ion competition, testing how adsorption behaves in the presence of other species of cations or anions.
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