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Development and characterization of hydrogels based on natural polysaccharides: Policaju and chitosan

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ABSTRACT

The development of hydrogels based on natural polysaccharides was investigated by preparing mixtures of policaju/chitosan at weight ratios of 1:4 and 2:3. Utilizing dynamic light scattering (DLS) techniques for these mixtures, an increase on the hydrodynamic particle radius was observed varying their pH from 3.0 to 12.0. Furthermore, a reduction of ζ -potential was also observed for the same pH interval. Following rounds of drying/hydration cycles at a specific pH value, hydrogel matrices were formed. The pore size distribution of these formed hydrogels was examined using scanning electron microscopy. Further FT-IR analyses confirmed a physical interaction between the polysaccharides policaju and chitosan. Swelling experiments revealed water uptake values, after 24 h of immersion in water, close to 270% for 1:4, and 320% for 2:3 hydrogels. Finally, rheological measurements were then conducted in order to confirm hydrogel viscoelastic features. These results indicate a promising road to biomaterials fabrication and biomedical applications.

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1. Introduction

Hydrogels are three-dimensional polymeric networks capable of absorbing large quantities of water or biological fluids. Due to their ability to absorb water, hydrogels attract interest both from the fundamental aspects of the swelling process as from their potential applications. It is known that hydrogels can be used to preserve cells, nutrients, drugs or proteins [1–3]. Many examples of application can be found, such as in contact lenses and materials for separation of biomolecules or cells, matrices for the immobilization of cells and devices for the controlled release of bioactive compounds [4–6], engineering and tissue regeneration [7,8], diagnostic and biological adhesions regulation [9], just to name a few.

Hydrogels are based on hydrophilic polymers that, when cross-linked, do not dissolve. In an aqueous environment, the hydrophilic groups of the polymer chains are hydrated generating a “network” structure for the hydrogels. The term network involves chemical or physical crosslinkage between the active groups of the polymer. Rheologically, aqueous solutions of hydrophilic polymers typically exhibit a Newtonian behavior when present in low concentrations. However,

since crosslinks among different polymer chains are introduced, the network thus obtained shows a viscoelastic and, sometimes, predominant elastic behavior [10]. In addition, hydrogels can be formulated in a variety of physical forms, including blends [11], micro- and nanoparticles [12], coatings and edible films [4].

Polysaccharides are natural long-chain polymers, linear or branched, nontoxic, biocompatible, biodegradable and of easy solubilization, and can form hydrogels or liquid crystals in solution [13]. Policaju is a polysaccharide obtained from *Anacardium occidentale* L. tree gum, abundant in the Northeast region of Brazil. Although the proportions of monosaccharides in the policaju vary depending on the seasonality of *A. occidentale* (source, age of the tree, time of exudation and climatic conditions), its chemical composition remains the same. It is an acidic polysaccharide complex with a molecular mass of 1.6×10^5 Da composed by a main chain of β -D-galactose (72%) connected by β -(1 \rightarrow 3) linkage -O6 substituted, having terminal residues of α -D-glucose (14%), arabinose (4.6%), rhamnose (3.2%) and glucuronic acid (4.7%) [14,15]. A standard property of policaju is its solubility in water forming a low viscosity solution, as well as its precipitation in organic polar solvents, such as ethanol. Additionally to its nontoxic, hydrophilic, biocompatible and biodegradable behavior, policaju has been reported in very important biomedical applications such as anti-tumoral, antiparasitic, emulsifying and suspending properties in pharmaceutical formulations and cicatrizing agent [16–19].

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Chitosan is a natural linear copolymer composed of glucosamine and *N*-acetylglucosamine units connected by $\beta(1-4)$ linkages. This polysaccharide is a deacetylated form of chitin, an abundant polysaccharide present in crustacean shells, arthropods and some fungi, being commercially produced with molecular mass between 3.8×10^3 and 2.0×10^4 Da [20]. Due to its hydrophilicity, biocompatibility, biodegradability and low toxicity, chitosan has been used as a biomaterial with wound healing ability, hemostatic properties and antimicrobial activity [21,22].

Both policaju and chitosan have already been reported on the literature as potential materials to form hydrogels; however, most studies promote chemical/structural modifications with the addition of active radicals (carboxyl or amino groups), or through different types of chemical crosslinks and physical interactions [23–27]. Nevertheless the use of unmodified policaju, in combination with chitosan, as a potential material to form hydrogel has not been widely explored in the literature [15,27–29].

Industrially, there is a growing interest in the formulation of mixed polysaccharide systems, leading to final products having specific properties and possible cost advantages. The aim of this work was developing and characterizing hydrogels containing policaju and chitosan without further modification in their chemical structure, and to evaluate an efficient method of preparation of these hydrogels. The hydrogels were characterized regarding their morphologies, particle size distribution, ζ -potential, water uptake, structure and rheological behavior.

2. Materials and methods

2.1. Materials

Polysaccharide from *A. occidentale* L. tree gum (collected from cashew in the South coast of Pernambuco, Brazil) was obtained according to the method described by Souza et al. [30] and termed policaju (POLI) (M_w ca. 1.1×10^5 g mol⁻¹ [15]). Chitosan (CHI) with $\geq 75\%$ deacetylation (M_w ca. 6.24×10^5 g mol⁻¹) was from Sigma (U.S.A.). All other reagents were of analytical grade and used without further purification.

2.2. Preparation of POLI–CHI hydrogels

The polysaccharide solutions of policaju (10% wt/vol) and chitosan (1% wt/vol) in 1.0% (vol/vol) lactic acid were previously prepared under agitation using a magnetic stirrer (500 rpm). Hydrogels with different proportions of policaju and chitosan (1:4, 2:3, 3:2 and 4:1, respectively) were prepared according to the modified method described by Abreu et al. [31]. Briefly, the chitosan pre-gel formation consisted of adding 0.1 mol/L CaCl₂ (1% vol/vol of the final volume of the desired mixture) to a beaker containing 1.0% (wt/vol) chitosan solution in 1.0% (vol/vol) lactic acid which was left stirring in a ultrahomogenizer (Ultra-Turrax, IKA, U.S.A) at 7000 rpm for 1 h. After that, the policaju solution 10.0% (wt/vol) in lactic acid 1.0% (vol/vol) was extruded through a syringe needle (27-gauge) at a flow rate of 1.0 mL/min and the mixtures, namely POLI–CHI pre-gel, were left stirring in Ultra-Turrax for 1 h. Then, the POLI–CHI pre-gel was set at different pH values (3.0–12.0) with 1.0 NaOH or HCl and stored under controlled temperature of 4 °C. The pre-gel of chitosan was used as control to observe any macro and microscopic change on the hydrogels obtained with the addition of policaju.

The different POLI–CHI pre-gels and chitosan pre-gel (blank) were distributed by glass Petri dishes (25 mL) and dried at 40 °C for 16 h. The thin film obtained was swollen with 5 mL of distilled water and dried again at 40 °C for 16 h and, from now on termed POLI–CHI hydrogel and chitosan hydrogel. This procedure contributes with the polymerization and activation of the matrix of the hydrogels for water uptake.

2.3. Hydrogel characterization

2.3.1. Dynamic light scattering (DLS) and ζ -potential

Dynamic light scattering (DLS) and ζ -potential (ζ) measurements were conducted on a ZetaSizer Nano ZS90 (Malvern Instruments, U.K.) for chitosan solution, policaju solution, and POLI–CHI pre-gel with different proportions of policaju/chitosan. The DLS cumulants analysis (30 scans) provides the characterization of a sample through the apparent Z-average hydrodynamic radius (R_h) for the particle size (μm) and polydispersity index (PDI), determined from the intensity of scattered light at 25 °C and a fixed scattering angle of 90°. The ζ -potential values were calculated using the Smoluchowski equation [32]. The measuring of the particle size was carried out in triplicate with samples analyzed in a period of 144 h, with intervals at 48 h for each analysis, in order to evaluate particle size distribution.

2.3.2. Water uptake

The effect of ionic strength and pH of the immersion solutions on water uptake (WU) capacity of the hydrogels was determined according to Paula et al. [33]. For the ionic strength studies hydrogel samples (ca. 1.0 g, each) were placed in a separate beaker containing NaCl (conc. 0.000, 0.056, 0.084 and 0.173 M). For the pH investigation, hydrogel samples (ca. 1.0 g, each) were poured in 200 mM buffered aqueous solutions of sodium citrate with pH 3.0 and 5.0; of sodium phosphate with pH 7.0 and 8.0, of Tris–HCl with pH 9.0, and of sodium carbonate with pH 11.0. After reaching equilibrium (over 48 h) the hydrogels were accurately weighed.

The WU of the different hydrogels was determined at temperature of 25 °C over the 48 h with the following equation:

$$\text{WU (\%)} = \left[\frac{W_w - W_D}{W_D} \right] \times 100 \quad (1)$$

where W_w is the weight of swollen hydrogel in water at each time and W_D is the weight of the dried hydrogel. Three samples of hydrogel were used for each measurement.

2.3.3. Scanning electron microscopy (SEM)

The SEM surface scans of the POLI–CHI pre-gels were conducted on a scanning electron microscope Quanta 200 FEG (FEI, USA) at an accelerating voltage of 10 kV under vacuum conditions. The samples were prepared onto coverslips attached to a support coated with a thin film of chromium and carbon to prevent the accumulation of static electric charge on the surface during electron irradiation and to avoid scanning faults and other image artefacts. The coverslips were dried naturally at room temperature (25 °C). For each sample internal matrix structure analysis, a cross-section of the swollen POLI–CHI hydrogels was lyophilized utilizing the cryofracture method.

2.3.4. FT-IR spectroscopy

FT-IR spectra of POLI–CHI pre-gels were taken on a spectrometer FT-IR Vertex 70 (Bruker, USA) in the range from 4000 to 450 cm⁻¹. Powdered samples were prepared with KBr to make pellets. To achieve a 2-cm⁻¹ resolution, 128 scans were made in each spectrum.

2.3.5. Rheological measurements—frequency sweeps, flow curves and temperature stability

All rheological measurements were carried out according to Pinheiro et al. [34] using a rheometer MCR301 (Anton-Paar, Austria). Initially, the experiments were conducted with the POLI–CHI pre-gels and a chitosan pre-gel. Afterward the tests were conducted in POLI–CHI and chitosan hydrogels. The measurements were obtained, in triplicate for each sample, in a parallel plate geometry (diameter: 25 mm) cell with a gap between plates of 1 mm. In order to determine the appropriate strain for linear viscoelastic regime, preliminary strain sweeps were conducted at different frequencies (0.1, 1.0 and 10.0 Hz) for variable strains ranging

from 0.1% to 10.0%. Frequency sweeps were carried out at 25 °C, in the 0.1–100 Hz range within the linear viscoelastic region. Moreover, the POLI–CHI hydrogels and the chitosan pre-gel were tested for temperature stability at oscillatory mode (frequency = 1.0 Hz) for a range of temperature from 0 to 70 °C, at a rate of 5 °C/min.

2.4. Statistical analysis

All results were expressed as mean \pm standard deviation, and analyzed with one-way analysis of variance (ANOVA) followed by a multi-parametric Tukey's post hoc test in GraphPrism® (GraphPad Software Inc., San Diego, CA). Statistical significance was established at $p < 0.01$.

3. Results and discussion

Different ratios of 10% (wt/vol) policaju/1% (wt/vol) chitosan (1:4, 2:3, 3:2, 4:1, respectively) were tested to obtain hydrogels, however the policaju/chitosan 3:2 and 4:1 ratios did not formed hydrogels. Probably, due to higher content of policaju and its high solubility in water, these proportions did not lead the oramation of thin films of hydrogels capable of absorbing water without dissolving. In view of these results, all the experiments were conducted only for the policaju/chitosan 1:4 and 2:3 ratios.

3.1. Hydrogel characterization

3.1.1. Dynamic light scattering (DLS) and ζ -potential measurements

The influence of pH (3.0–12.0) on the ζ -potential (mV) and particle size (μm) was evaluated by DLS for policaju (10% wt/vol) and chitosan (1% wt/vol) solutions and POLI–CHI pre-gels (1:4 and 2:3). In a range of pH 3.0–12.0, the ζ -potential of policaju solutions showed values between +5.5 and -1.8 mV, considered a neutral range, which means that the carboxyl group content of 6.3% [14] of this polysaccharide was inefficient to present a negative charge (Fig. 1A). The polysaccharides may be constituted either by polycations or by polyanions, depending on their functional group, and may also be neutral, which is the case of different types of polysaccharides with a higher content of mannose and galactose units, with ζ -potential values between -13.7 and -2.1 mV [35].

Chitosan solutions presented positive ζ -potential values, ranging between +42.8 and +1.65 mV when the pH increased from 3.0 to 12.0 and this can be explained because the chitosan is a polycation with a pKa of about 6.50 and has amino groups which can easily be positively charged [36]. These results are in agreement with the ζ -potential values obtained by Yu et al. [37] and Carneiro-da-Cunha et al. [35], which observed a reduction of ζ -potential of chitosan solutions, in a range of +60.0 and -0.1 mV, with the increase of the pH value from 2.5 to 11.0. The ζ -potential values obtained by these authors are higher than those obtained in the present work. This is possibly due to the higher deacetylation degree of the chitosan used by those authors, when compared to the one used in the work reported here. In fact, it is known that the higher the deacetylation degree, the higher the content of amine groups positively charged on the surface of the chitosan particles.

All POLI–CHI pre-gels (1:4 and 2:3) showed a decrease of ζ -potential from +37.2 to -3.2 mV with 1:4 ratio and from +18.1 to -0.4 mV with 2:3 ratio, with the pH increase. The relative charge beyond the hydrodynamically stagnant layer of POLI–CHI pre-gels (1:4 and 2:3) showed that there were significant differences in the resulting ζ -potential with the increase of policaju content (Fig. 1A).

As a general rule, suspensions with ζ -potential above 60 mV (absolute value) show excellent stability; above 30 mV, suspensions are physically stable; below 20 mV, they are of limited stability; and below 5 mV, they undergo pronounced aggregation [38]. The ζ -potential values obtained for POLI–CHI pre-gels at pH 5.0 are in the limit of physical stability (Table 1), showing positive values of ζ -potential (+13.30 \pm 0.62 mV)

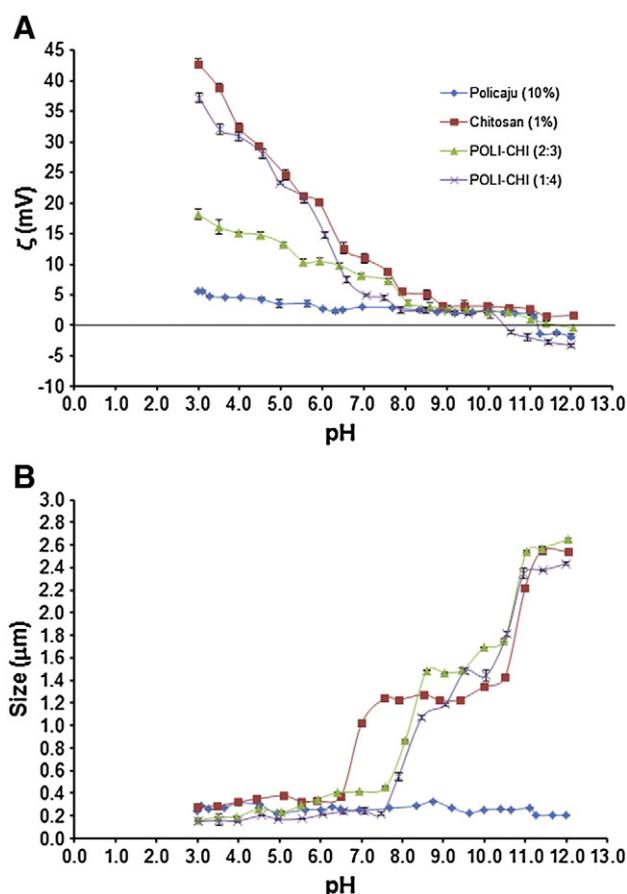


Fig. 1. (A) The pH dependent ζ -potential of policaju, chitosan and POLI–CHI pre-gels 1:4 and 2:3. (B) DLS measurements of policaju, chitosan and POLI–CHI pre-gels 1:4 and 2:3. Each data point is the average of three determinations and error bars show the standard deviation.

for 2:3 POLI–CHI pre-gel with high policaju content and also positive for 1:4 POLI–CHI pre-gel with low policaju content (+23.27 \pm 0.25 mV). The pre-gels with higher ζ -potential values have a higher charge density of the amino groups on the surface and are stable, as in the case of pre-gel obtained with higher chitosan content (1:4). These changes on chitosan charge, and on reduction of POLI–CHI pre-gels charge were similar to those observed in alginate and chitosan pre-gels reported by Abreu et al. [31] and in nanogels prepared by electrostatic self-assembly of chitosan and ovalbumin reported by Yu et al. [37]. Of note, the association of chitosan with other natural or synthetic polymers is a convenient and effective way to improve its physical properties for practical applications [39,40]. Since chitosan and policaju are polyelectrolytes, it is possible to obtain ionic complexes through hydrogen bonding or electrostatic interactions [41], resulting in interpenetrating polymer network. Multivalent cations, such as Ca^{2+} , can be coordinated with carboxylate groups present in the hydrogel [42] and associated with $-\text{OH}$ groups present in the polysaccharide [43], which results in the formation of polyanionic-cationic complexes resembling a stabilized isotropic gel network.

Table 1

DLS measurements of the policaju, chitosan and POLI–CHI pre-gels 1:4 and 2:3 at pH 5.0.

Solutions	ζ (mV)	Size (μm)	PDI
Policaju 10%	3.54 \pm 0.61	0.224 \pm 0.004	0.578 \pm 0.017
Chitosan 1%	24.57 \pm 0.83	0.378 \pm 0.006	0.500 \pm 0.056
POLI–CHI (1:4)	23.27 \pm 0.25	0.168 \pm 0.009	0.257 \pm 0.027
POLI–CHI (2:3)	13.30 \pm 0.62	0.233 \pm 0.008	0.374 \pm 0.007

Fig. 1B shows that the particle size of polycaju solutions was almost constant at all pH values (3.0–12.0), whereas chitosan solutions showed a slight increase at the pH range of 3.0–5.0, remaining constant from pH 5.0 to 6.5. When pH values exceeded 6.5, the precipitation occurred immediately due to the aggregation behavior of the particles of chitosan observed through the formation of turbidity and confirmed by increase of the particles sizes.

Aggregation of polysaccharide solutions can be related with the charge of the polysaccharide, where different charges will induce different behaviors [35]. This phenomenon of aggregation in chitosan solutions at neutral and basic pH values is related to the presence of hydroxyl and amino groups in chitosan's structure [44]. All the POLI–CHI pre-gels with 1:4 and 2:3 ratio showed a slow increase of particles size in the pH range of 3.0–7.5, from 0.158 to 0.217 μm and from 0.149 to 0.455 μm , respectively, while at pH 7.5 the particle size of pre-gel 2:3 ratio was twice of that found for the pre-gel 1:4 ratio. For pH values above 7.5, the charges and the solubility of chitosan ($\text{pKa} \leq 6.5$) decrease rapidly, preventing the formation of dispersible pre-gels and leading to precipitates, confirmed by the turbidity of the material in the tubes and DLS measurements.

At pH values ≤ 5.0 the particle size of the pre-gels was lesser or equal to the particle size of the constituents of the polysaccharide solutions (Table 1). This may have been generated by the ultra-homogenization process since the pre-gels undergo a physical extrusion in the Ultra-Turrax, thus reducing particle size. The use of ultra-homogenization process has been reported to reduce particle size of macromolecular solutions [45]. Information concerning size homogeneity of the particles within a sample is provided by DLS measurements in terms of polydispersity index (PDI).

The PDI describes the deviation of the measured autocorrelation function from that of a dispersion of monodisperse spheres with the same diameter. PDI values greater than 0.5 indicate a very broad particle-size distribution which is probably not suitable for DLS evaluation [46,47]. Up to pH 5.0, lower PDI values were obtained for the POLI–CHI pre-gels (1:4 and 2:3), suggesting that there is a reduced

dispersion of average particle size for each pre-gel (Table 1). Therefore, in view of the results obtained for ζ -potential and particle size, pH 5.0 was chosen in this work to produce stable POLI–CHI hydrogels.

3.1.2. Water uptake

According to the method describe before, POLI–CHI pre-gels were used to obtain POLI–CHI hydrogels, and the swelling rate of POLI–CHI (1:4 and 2:3) and chitosan hydrogels was investigated by water uptake (WU) capacity (Fig. 2). Chitosan hydrogel swelled substantially over the course of the first 2 h when reached saturation and remained constant. In contrast, POLI–CHI hydrogels reached saturation with 24 h of immersion in water and remained constant until the final of the experiment (48 h). POLI–CHI hydrogels 1:4 and 2:3 ratios swelled up to 270% and 320%, i.e. it absorbed a mass of water originating a hydrogel with 3.7 and 4.2 times its dry weight, respectively. These results show that higher the polycaju content, more cross-linked is the hydrogel. Furthermore, it was observed that POLI–CHI hydrogels 1:4 or 2:3 demonstrated to be extremely soft due to the high absorption of water.

No statistically significant differences in the WU values were found for the POLI–CHI hydrogels with 48 h of immersion in water (Fig. 2A) when compared to the corresponding values after 24 h of immersion in water (270% and 320% for 1:4 and 2:3 ratios, respectively).

The effect of ionic strength and pH of immersion solution on the water uptake capacity (WU) of the hydrogels was also evaluated. The WU was dependent on the ionic strength and pH of immersion solution. Regarding the ionic strength, an increase of WU capacity was observed with the increase of ionic strength (NaCl 0.000, 0.056, 0.084 and 0.173 M); besides there was no significant difference observed for POLI–CHI hydrogel 2:3 ratio. The WU maximum values of the POLI–CHI 1:4 and 2:3 ratios were 295% and 330%, respectively, with 48 h of immersion in the 0.173 M NaCl solution (Fig. 2B). The pH of the aqueous solution was varied between 3.0 and 11.0, using different buffered systems according to their pKa value (Fig. 2C). The results showed a maximum value of WU for both POLI–CHI hydrogels 1:4 and 2:3 ratios at pH 3.0 (300% and 380%, respectively), decreasing with the increasing of

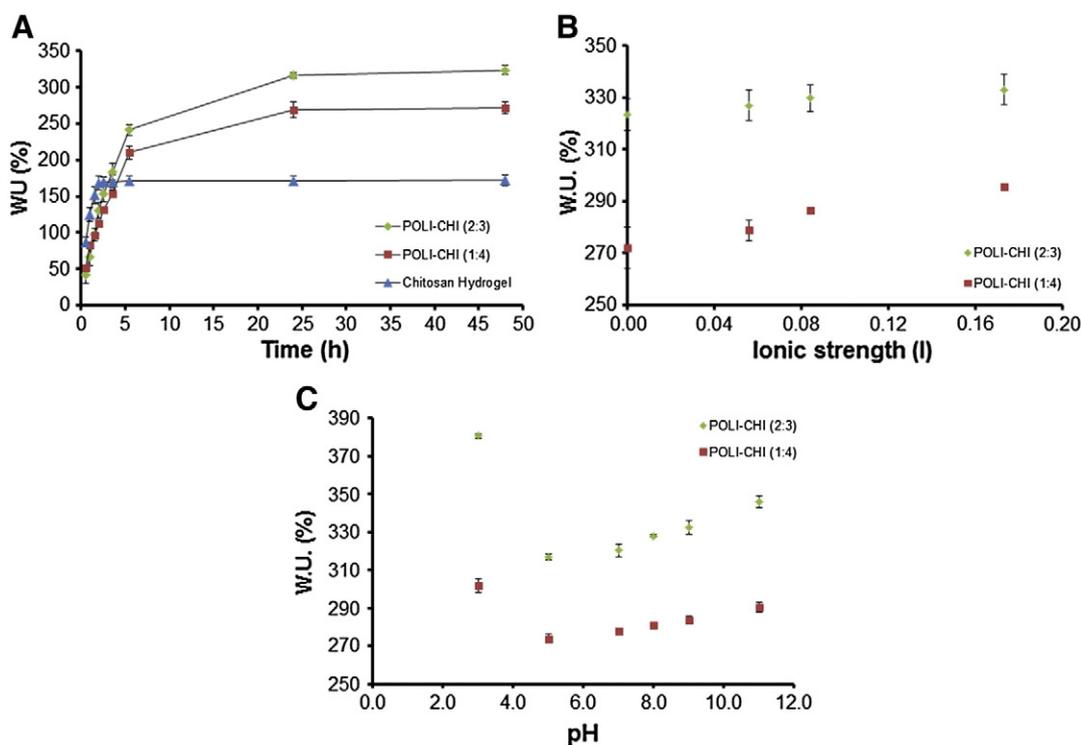


Fig. 2. (A) Water uptake by the POLI–CHI hydrogels 1:4 and 2:3 and chitosan hydrogel at pH 5.0 for 48 h in immersion of water. (B) Ionic strength effect on water uptake capacity by POLI–CHI hydrogels 1:4 and 2:3. (C) pH effect on water uptake capacity by POLI–CHI hydrogels 1:4 and 2:3. Each data point is an average of three experiments and the error bars show the standard deviation.

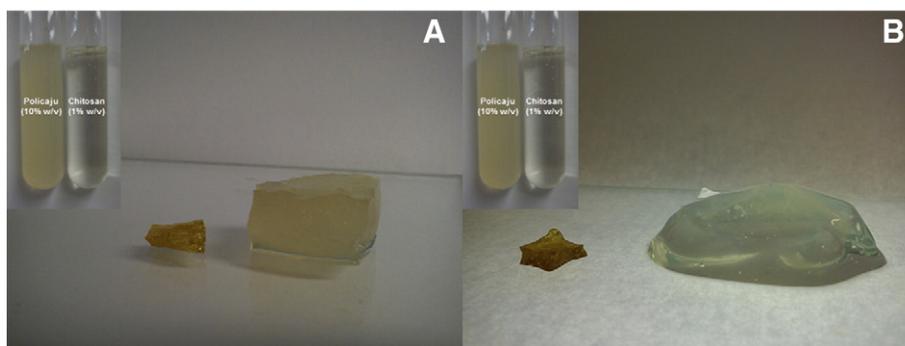


Fig. 3. Dry and swollen POLI-CHI hydrogels after a period of 48 h. (A) 1:4. (B) 2:3. Insert shows polysaccharide solutions of 10% (wt/vol) policaju and 1% (wt/vol) chitosan.

pH from 3.0 to 5.0. In an interval pH range of 5.0–11.0, an increase on WU capacity reaching values of 285% and 340% at pH 11.0 for POLI-CHI hydrogels 1:4 and 2:3, respectively, was verified. The maxima in WU capacity could be explained by the state of ionization of the main chemical groups in the mixtures; the groups responsible for maintaining the chain expansion and elongation: at the pH 3.0 maximum, the chitosan (NH^{+3}) group, and at the pH 11.0 maximum the policaju (COO^-) group. Similar results were reported in swelling studies for chitosan/cashew gum physical gels [33].

Superabsorbent hydrogels based on mixtures of different polysaccharides, with high WU values have been also reported in the studies by Guilherme et al. [23] and Chang et al. [48] who managed WU values for hydrogels around 325% (acrylamide/cashew gum) and 100% (cellulose/carboxymethylcellulose) of their dried weight, respectively. Fig. 3 shows POLI-CHI hydrogels 1:4 and 2:3 swollen with 48 h and the inserted picture shows the polysaccharide solutions of 10% (wt/vol) policaju and 1% (wt/vol) chitosan; the colors of dried POLI-CHI hydrogels 1:4 and 2:3

and swollen POLI-CHI hydrogel 1:4 are similar to the color of policaju solution. The POLI-CHI hydrogel 2:3 was brighter than the 1:4 hydrogel because of the higher content of water absorbed.

3.1.3. POLI-CHI hydrogels morphology

SEM results (Fig. 4) show a cross section in cryofracture of the matrix of POLI-CHI hydrogels 1:4 and 2:3. The hydrogel matrices of the samples exhibited a macroporous architecture with pore size distribution, being more uniform and smaller for hydrogel 1:4, with sizes of approximately $6\ \mu\text{m}$ (Fig. 4A and B). This uniform pore distribution for hydrogel 1:4 agrees with PDI values shown in Table 1. It can be observed that an increased amount of policaju content led to changes in the morphology of POLI-CHI hydrogel; this is evident in the higher number of pores as well as in the asymmetrical pore size distribution observed for hydrogels 2:3, with pore sizes peaking at $72\ \mu\text{m}$ (Fig. 4C and D).

This higher porosity distribution for hydrogel 2:3, agrees that this hydrogel leads to more swelling and lowers the stiffness in comparison

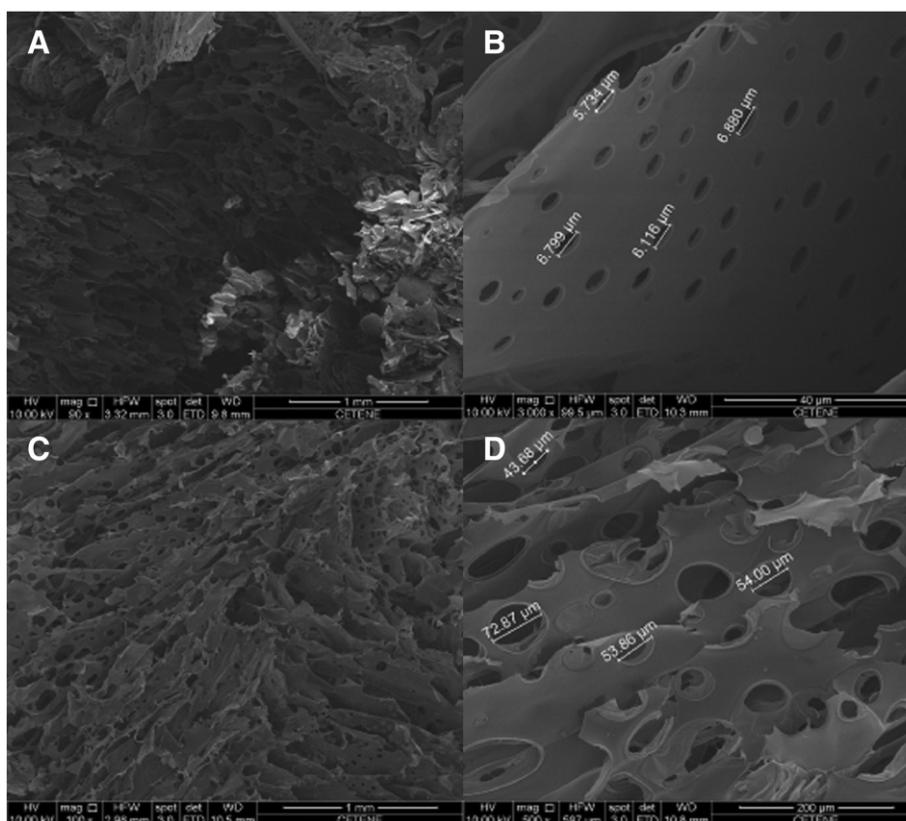


Fig. 4. SEM micrographs of cross-section in cryofracture of POLI-CHI hydrogels. (A and B) 1:4 (90 \times and 3000 \times , respectively). (C and D) 2:3 (100 \times and 500 \times , respectively).

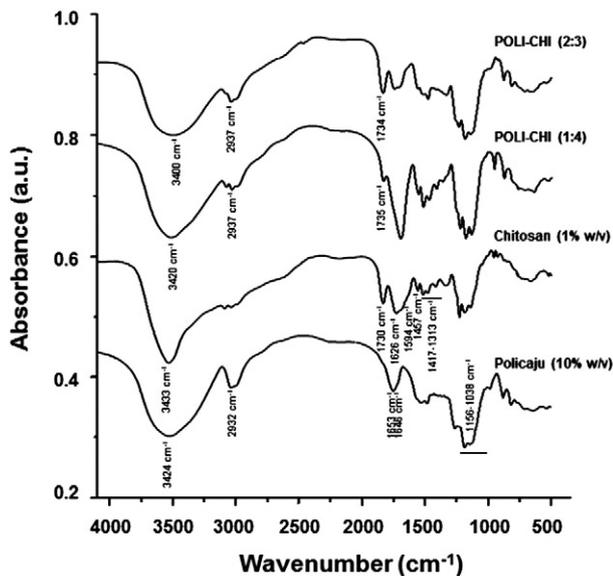


Fig. 5. FT-IR spectra of 10% (wt/vol) polycaju, 1% (wt/vol) chitosan, and POLI-CHI pre-gels 1:4 and 2:3 at pH 5.0.

with the hydrogel 1:4. These results shown that increasing the polycaju content in POLI-CHI formulation was capable of changing the matrix of hydrogels and add new characteristics which raised new possible biotechnological applications.

3.1.4. FT-IR analyses

The interaction between the polysaccharides can be observed through changes in the vibration modes of the main groups observed by infrared spectroscopy (Fig. 5). Chitosan of 1.0 % (wt/vol) shows a broad absorption at 3433 cm^{-1} from stretch vibrations of $-\text{OH}$ and $-\text{NH}_2$ groups. Carbonyl groups ($\text{C}=\text{O}$) were detected at 1730 cm^{-1} . Their principal vibration modes are asymmetrical and symmetrical bending NH_3^+ (amide I and II) vibrations at 1628 , 1594 and 1457 cm^{-1} . Other important stretching vibrations of $-\text{CH}_2-$ from pyranosidic structures were observed at 1417 – 1313 cm^{-1} [28,29].

The weak band at 1653 cm^{-1} in the infrared spectroscopy of 10% (wt/vol) polycaju was assigned to the $\text{C}=\text{O}$ group from glucuronic acid, present at 6% levels in the original gum. A broad band at 3424 cm^{-1} due to the stretching vibration of $\text{O}-\text{H}$, a small peak at 2932 cm^{-1} , attributed to the $-\text{CH}$ stretching vibration, and absorption at 1646 cm^{-1} , due to $\text{O}-\text{H}$ scissor vibrations from bound water molecules were observed. Strong peaks at 1156 – 1038 cm^{-1} were also observed, due to stretching vibrations of $\text{C}-\text{O}-\text{C}$ from glucosidic bonds and $\text{O}-\text{H}$ bending from pyranosidic structures in agreement with the FT-IR of the nanoparticles [27] and beads [29] obtained by mixing of polycaju and chitosan.

The POLI-CHI complex formation was evidenced by the sharpening of the bands from the two polysaccharides. Amine ($-\text{NH}_2$) and carboxyl ($\text{C}=\text{O}$) groups from chitosan and the $-\text{CH}$ group from polycaju were detected in POLI-CHI pre-gels (1:4 and 2:3); however, the bands were shifted, which demonstrate the presence of molecular interactions between these two polymers. Physical interactions as Van der Waals forces and hydrogen bonds may play an important role in this hydrogel composition, suggesting a physical hydrogel type. Ionic interactions can be discarded because of the absence of ionic groups in polycaju structure

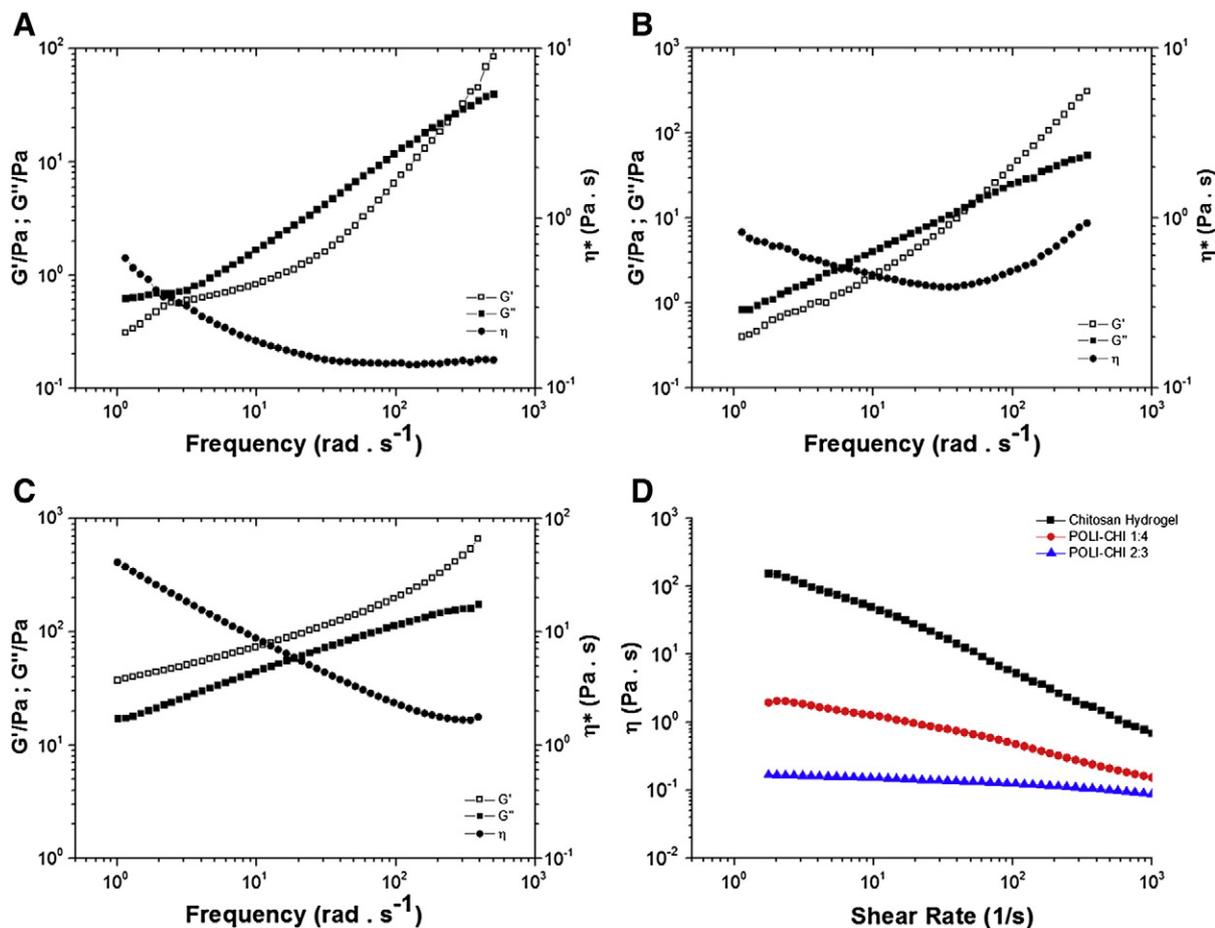


Fig. 6. Mechanical spectra of POLI-CHI mixtures and chitosan hydrogel, at $25\text{ }^\circ\text{C}$. (A) 2:3. (B) 1:4. (C) Chitosan hydrogel. (D) Apparent viscosity versus shear rate of 1:4 and 2:3 POLI-CHI mixtures and chitosan hydrogel.

available to interact with the amino groups of chitosan detected in the pre-gels spectra.

3.1.5. Dynamic shear properties

The rheological behavior of POLI–CHI (1:4 and 2:3) and chitosan pre-gels was evaluated. The rheometry of the mixed polysaccharide pre-gel solutions showed the typical liquid–solid behavior of macromolecular solutions: at low frequencies the viscous modulus, G'' dominated over the elastic modulus, G' , i.e., the system showed liquid-like features. A crossover frequency for the two moduli was evident. After this value the elastic response prevailed. Furthermore, the cross-over frequency decreased as the policaju concentration decreased in the mixture.

In order to evaluate the gelation of the POLI–CHI hydrogel candidates, oscillatory dynamic measurements were carried out for the highest swollen hydrogels over a frequency range of 1–100 Hz. Fig. 6A–C shows mechanical spectra of POLI–CHI mixtures (1:4 and 2:3) and chitosan hydrogel. The chitosan hydrogel exhibited a predominant elastic behavior, with storage modulus (G') greater than loss modulus (G''), for the entire frequency interval investigated; this behavior contrasts with the one observed for the pre-gel of chitosan (liquid-like behavior, results not shown).

The swollen POLI–CHI (1:4 and 2:3) hydrogel candidates showed typical viscoelastic behavior. Nevertheless, there was a reduction in the crossover frequency between the G' and G'' compared to the POLI–CHI pre-gels (results not shown), indicating that gelification partially occurred. It should be stressed that the hydrogel mixtures used were at the highest swelling ratios. Lower amounts of retained water should increase the elastic contribution from the polymeric structure.

POLI–CHI swollen hydrogels (1:4 and 2:3) showed an increase in the complex viscosity values with the increase in frequency, being this increase a function of the policaju content in the mixture (Fig. 6A and B). The higher the policaju content, the lower the complex viscosity values. This indicates that POLI–CHI hydrogel 1:4 has a higher

interaction between the polysaccharides than POLI–CHI hydrogel 2:3. The role of policaju concentration is best seen in Fig. 6D, showing the magnitude of apparent viscosity versus shear rate for 1:4 and 2:3 POLI–CHI hydrogels and chitosan hydrogel.

During the preparation stage, it was found that temperature affects the physical structure of hydrogels. Fig. 7 shows mechanical spectra of POLI–CHI hydrogels and chitosan hydrogel, conducted in oscillatory mode, as a function of temperature (0–70 °C). For the POLI–CHI 2:3 hydrogel samples tested, G'' was greater than G' , showing a steady reduction with an increase in temperature with no crossovers (Fig. 7A). Assuming no substantial evaporation had occurred, it was observed that at 62.7 °C for POLI–CHI 1:4 hydrogel (Fig. 7B) and 57.2 °C for chitosan hydrogel (Fig. 7C), G' crossed over G'' , indicating that an ultimate gelling structure has been reached. An increase in complex viscosity values was observed accordingly indicating a greater interaction of the polysaccharides involved and an increase in the organization of these hydrogel networks when compared to the POLI–CHI 2:3 hydrogel samples.

Since the hydrogels are highly swollen, the latter results could also be interpreted as due to variations in water content as a function of temperature.

4. Conclusion

Hydrogels of policaju and chitosan were obtained successfully using an ultra-homogenization process of direct injection and extrusion with an ULTRA-Turrax. At pH 5.0 it was possible to obtain stable polysaccharide solutions with low PDI values and small particles. The matrix structure of POLI–CHI hydrogels showed a network with pores of different sizes; networks more uniform for POLI–CHI 1:4 hydrogels than POLI–CHI 2:3 hydrogels. FT-IR analysis confirmed the existence of physical interactions between the polysaccharides involved and rheological measurements showed a decrease in complex viscosity with the increase of policaju content. Temperature seems to play an important

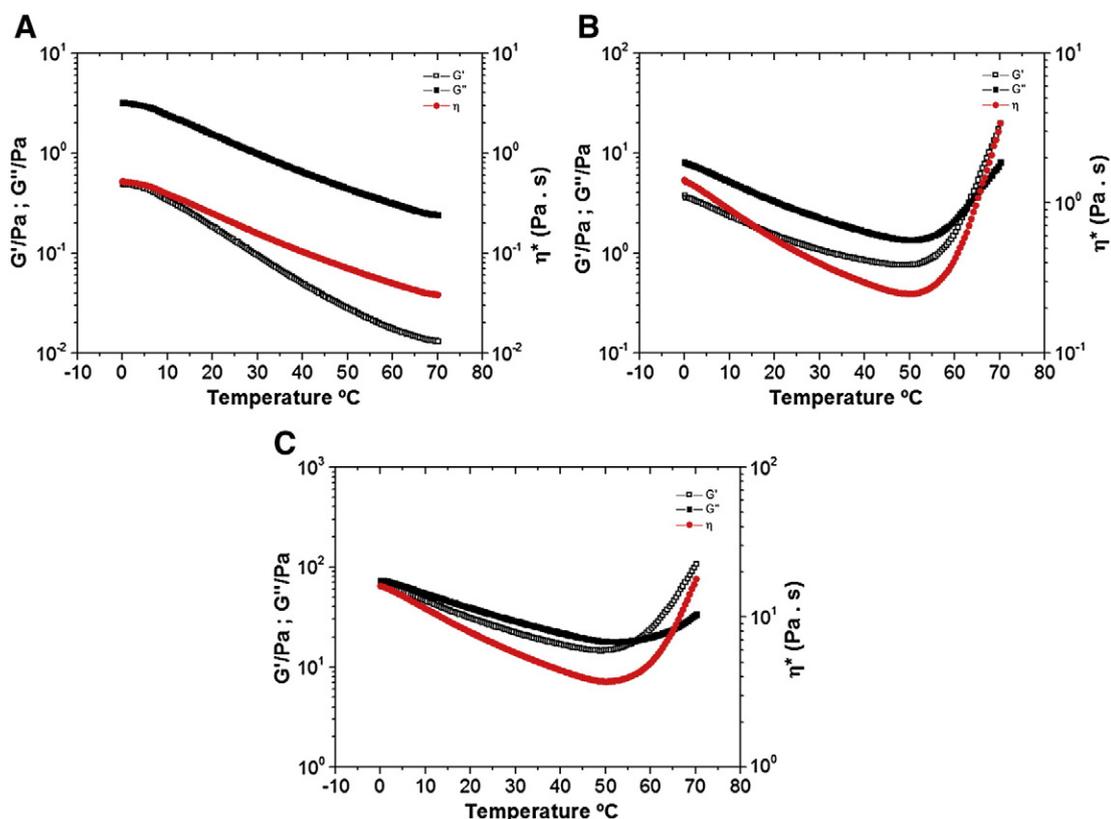


Fig. 7. Temperature influence in the hydrogels matrix: (A) 2:3, (B) 1:4, (C) chitosan hydrogel.

role in hydrogel formation. Above 57 °C it was possible to polymerize POLI–CHI hydrogels.

Dried POLI–CHI hydrogels swelled until 4.2-fold their initial weight. An increase of policaju content induced a higher water uptake; therefore this increase of policaju content also resulted in weaker hydrogels. The biological properties of polysaccharides involved and the features presented by POLI–CHI hydrogels make them attractive for applications in biotechnology, especially in biomedical, cosmetic and pharmaceutical industries. Finally, the uniform porosity for hydrogel 1:4 suggests that it might be suitable for biotechnological applications such as drug-controlled release. Conversely, the high swelling and fluid composition for hydrogel 2:3 suggest its possible use in cosmetic and medical industry as a topical gel.

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