Development and characterization of a new hydrogel based on galactomannan and κ-carrageenan

Paulo A.G. Soares\textsuperscript{a, b}, José R.P. C de Seixas\textsuperscript{a}, Priscilla B.S. Albuquerque\textsuperscript{a, b}, Gustavo R.C. Santos\textsuperscript{c}, Paulo A.S. Mourão\textsuperscript{c}, Wilson Barros Jr.\textsuperscript{d}, Maria T.S. Correia\textsuperscript{a}, Maria G. Carneiro-da-Cunha\textsuperscript{a, b, \*}

\textsuperscript{a} Departamento de Bioquímica, Universidade Federal de Pernambuco (UFPE), Av. Prof. Moraes Rego, s/n, Cidade Universitária, CEP: 50670-420 Recife, PE, Brazil
\textsuperscript{b} Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Campus Universitário, s/n, Cidade Universitária, CEP: 50670-901 Recife, PE, Brazil
\textsuperscript{c} Laboratório de Tecido Conjuntivo, Hospital Universitário Clementino Fraga Filho and Programa de Glicobiologia, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro (UFRJ), Ilha do Fundão, CEP 21941-913 Rio de Janeiro, RJ, Brazil
\textsuperscript{d} Departamento de Física, Universidade Federal de Pernambuco (UFPE), Av. Prof. Luiz Freire s/n, Cidade Universitária, CEP 50670-901 Recife, PE, Brazil

\textbf{A R T I C L E  \ I N F O}

\textbf{Article history:}
Received 28 April 2015
Received in revised form 14 August 2015
Accepted 14 August 2015
Available online 20 August 2015

\textbf{Keywords:}
Hydrogel characterization
Cassia grandis
Vegetal polysaccharides
Rheology
NMR

\textbf{A B S T R A C T}

A new hydrogel based on two natural polysaccharides was prepared in aqueous medium with 1.7\% (w/v) galactomannan (from \textit{Cassia grandis} seeds) and different concentrations of κ-carrageenan (0.3, 0.4 and 0.5\% w/v), CaCl\textsubscript{2} (0.0, 0.1 and 0.2 M) and pH (5.0, 5.5 and 6.0), using a full factorial design based on rheological parameters. The best formulation was obtained with 1.7\% (w/v) galactomannan and 0.5\% (w/v) κ-carrageenan, containing 0.2 M CaCl\textsubscript{2} at pH 5.0. Nuclear magnetic resonance and scanning electron microscopy where used in order to characterize the hydrogel formulation. A shelf life study was carried out with this formulation along 90 days-period of storage at 4 °C, evaluating pH, color, microbial contamination and rheology. This hydrogel showed no significant changes in pH, no microbial contamination and became more translucent along the aging. Analyses by nuclear magnetic resonance and rheology showed a larger organization of the polysaccharides in the hydrogel matrix. The results demonstrated that this hydrogel was stable with possible applications in medical and cosmetic fields.

\copyright 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Hydrogels are macromolecular networks formed from natural or synthetic hydrophilic polymers that can absorb and retain a significant amount of water without dissolving (Lefnaoui & Moulai-Mostefa, 2014; Ahmed, 2013). The hydrogel resistance to dissolution arises from cross-links between network chains, while their ability to absorb water arises from hydrophilic functional groups distributed along the polymeric backbone (Ahmed, 2013). Due to the combination of solid-like properties from the macromolecular network and liquid-like properties from the absorbed water, hydrogels exhibit viscoelasticity resembling that of biological tissue. In addition to these rheological properties, their high biocompatibility have attracted attention in drug delivery applications (Da-Lozzo et al., 2013), pharmaceuticals (Laurens et al., 2014), biomedical applications (Kenawy, Kamoun, Mohy Eldin, & El-Meligy, 2014), tissue engineering and regenerative medicine (Huang, He, & Wang, 2013), diagnostics (Feyzkhanoa et al., 2014), wound dressing (De Cicco et al., 2014), separation of biomolecules or cells (Ahmad et al., 2014), barrier materials to regulate biological adhesions (Zhang et al., 2011), and biosensors (Liu et al., 2015).

When forming hydrogels, polysaccharides of natural origin have improved properties in comparison with synthetic polymers (Manjanna, Pramod Kumar, & Shivakumar, 2010). Among natural polymers, there are the gelling polymer agents as carrageenans, and also thickening polymers, such as galactomannans, that, although not producing network real cross-links, possess gel properties above some specific concentration. Kappa-carrageenan (κC) is obtained by alkaline extraction (or by modification of other carrageenans) from red seaweed (\textit{Rhodophyceae}) (Prajapati, Maheriya, Jani, & Solanki, 2014). Without chemical modifications, it has the ability to form a strong three-dimensional network in presence of...
counterions (K⁺ and Ca²⁺) when compared with other types of carrageenans (lambda-carrageenan). κC is composed of alternating residues of 3-linked β-D-galactopyranose 4-sulfate and 4-linked 3,6-anhydro-α-D-galactopyranose residues (Prajapati et al., 2014; Pinheiro et al., 2011). Due to its high hydrophilicity, mechanical strength, biocompatibility and biodegradability, this polymer has been mostly used in food industry as gelling, stabilizing and thickener agent (Dima, Cotărlet, Alexe, & Dima, 2014). Nevertheless it has also been used to reduce or to eliminate toxicity in biomedical applications (Muhamad, Fen, Hui, & Mustapha, 2011).

Galactomannans are widespread polysaccharides commonly extracted from the endosporem of numerous seed plants (particularly the Leguminosae) which develop energy-reserve and hydration functions (Prajapati et al., 2013). The galactomannan extracted from Cassia grandis seeds have a core contributed by 4-linked β-D-mannopyranose with a repetitive single branch of α-D-galactopyranosyl units linked to position 6 of the central chain (Albuquerque et al., 2014). Due to its high molecular weight, water solubility, non-ionic character, biocompatibility and gel properties in higher concentrations, this polysaccharide can be used in food, pharmaceutical, biomedical, cosmetic, textile and paper industries, especially as an emulsion stabilizer (Albuquerque et al., 2014; Pinheiro et al., 2011).

Various hydrogels formulated from natural polymers have been used in industry. However, it has become clear that hydrogels consisting of single polymer arrangements cannot meet entirely the demands in terms of both properties and performance (Ahmed, 2013). In order to improve gel properties, new hydrogels based on two or more polymers have been developed in the last years (Jang et al., 2014; Diao, Li, Xiao, Duan, & Xu, 2014; Soares et al., 2014; Dash, Foston, & Ragauskas, 2013; Martins et al., 2012). Sometimes, chemical modification in involved polymers, chemical crosslinks and/or physical interactions is necessary. Such mixed systems include polymer blends, copolymer, interpenetrating or semi-interpenetrating polymer networks, with desirable properties (Liu et al., 2014; Lv et al., 2014; Vudung et al., 2014; Bhattacharyya & Ray, 2014).

Along the years, the use of rheology for characterization of viscoelastic properties in the production and application of polymeric materials such as gels and hydrogels has been essential (Albuquerque et al., 2014; Jang et al., 2014; Dash et al., 2013; Sandolo, Matricardi, Alhaique, & Coviello, 2007). The hydrogels behavior during flow and applied stress are important quality control parameters responsible for maintaining the products superiority and increase its shelf life. In this work we have developed a new hydrogel based upon optimized rheological characteristic of a mixture of κ-carrageenan and galactomannan from C. grandis seeds. This optimization was achieved via a full factorial design 2³ by which changing pH and both κC and cation concentrations, the hydrogels formed were rheologically tested. In addition to a primary characterization of the new hydrogel, shelf life studies were also carried out to guarantee its features during storage, making it viable in biomaterial industry applications.

2. Material and methods

2.1. Material

The pods of C. grandis were collected at the rural zone of Perambuco State, in the city of Angelim (Brazil), in July 2011. Ethanol 99.8%, acetone PA, NaCl and phenol were obtained from Vetec Fine Chemicals Ltda. [Brazil]. κ-Carrageenan was purchased from Sigma Aldrich (U.S.A.). All other chemicals were of analytical grade.

2.2. Extraction of the galactomannan from C. grandis seeds

The galactomannan from C. grandis seeds was extracted and purified according to the method described by Albuquerque et al. (2014). Briefly, dried seeds were boiled in distilled water 1:5 (w/v) at 100 C for 1 h and maintained for 18 h at 25 C to facilitate removal of the hull. The endosperm plus germ, were triturated in a blender with 0.1 M NaCl 5% (w/v) at 25 C, filtered and precipitated with 46% ethanol 1:3 (v/v) for 24 h. The precipitate was filtered again, followed by a wash with 100% ethanol 1:3 (v/v) and twice with acetone P.A. 1:3 (v/v), with filtrations between each washing. The precipitated galactomannan obtained was dried in an oven at 100 C and finally pulverized and kept in a dry place until further use.

2.3. Hydrogel preparation

Stock solutions of κC in different concentrations 0.3, 0.4 and 0.5% (w/v) and galactomannan (1.7% w/v) were prepared. A known volume of κC was added into a beaker in addition to a known concentration of CaCl₂. The solution was maintained under magnetic stirring (850 rpm), at 50 C during 20 min. The addition of CaCl₂ (1.0% (v/v) of the final volume of desired mixture) was necessary to ignite a crosslinking process. In order to evaluate the latter process, hydrogel samples with three CaCl₂ concentrations were actually utilized: 0 M, 0.1 M, and 0.2 M. After that, a well-known volume of galactomannan solution, was added into the beaker containing the κC+CaCl₂ solution at 1 ml/min flow rate, using a needle syringe (27G). The mixture was left stirring under 850 rpm at 50 C, during additional 30 min. Several samples were prepared from the mixture adjusting their pH to 5.0, 5.5 or 6.0 using a 1 M HCl solution. These final samples, now named hydrogels, were distributed in a cell culture plate (6-well plate) and stored at 4 C.

2.3.1. Experimental design and statistical analysis

The best hydrogel mixture was selected by monitoring the influence of the following independently controlled parameters: κ-carrageenan (κC) and CaCl₂ (Cl) concentrations, pH onto the measured complex viscosity (η*), viscous–elastic moduli ratio (G'/G″ = tan δ), and the phase transition temperature (Tδ). The study was conducted employing a 2³ full factorial design (see Supplementary material), in addition to a central point in quadruplicate to allow for the estimation of pure experimental error. The results were statistically validated by an analysis of variance (ANOVA) at a significance level of p < 0.05. All statistical and graphical analyses were carried out with the Statistica 8.0 program (SatSoft Inc., 2008, Tulsa, OK, USA).

2.4. Hydrogel characterization

2.4.1. Nuclear magnetic resonance spectroscopy

One dimensional (1D) ¹H NMR spectra of the polysaccharides and hydrogels were recorded using a Bruker DRX 600 MHz apparatus with a triple resonance probe, as described previously by Tovar et al. (2012). Approximately 5 mg of each sample was dissolved in 0.5 ml of 99.9% deuterium oxide (Cambridge Isotope Laboratory, Cambridge, MA, USA). The spectra were recorded at different temperatures (25 C to 55 C) with HOD (deuterated water exhibiting a peak due to exchange with residual H₂O) suppression by presaturation. For NMR spectra, 32 scans were recorded using an inter-scan delay equal to 1 s and the chemical shifts were displayed relative to external trimethyl-silylpropionic acid at 0 ppm for ¹H. The range of temperature was made in order to observe the temperature influence on the hydrogel matrix.
2.4.3. Rheological measurements

All rheological measurements were carried out, in triplicate for each sample, according to Pinheiro et al. (2011) using a rheometer MCR301 (Anton-Paar, Austria), 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 within a 0.1–100 Hz range, maintaining a linear viscoelastic regime (0.2% strain deformation). Moreover, the hydrogel was tested for temperature stability (see Supplementary material) at oscillatory mode (frequency = 1.0 Hz) for a range of temperature from 0 to 70 °C, at a rate of 5 °C/min increase. Unless explicitly mentioned, all rheological measurements were conducted at 25 °C.

2.4.4. Scanning electron microscopy (SEM)

The SEM surface scans of the hydrogel were conducted on a scanning electron microscope EVO LS15 (ZEISS, Germany) with an accelerating voltage of 10 kV under vacuum conditions. The hydrogel samples were prepared 24h before measurements. For each sample internal matrix structure analysis, a cross-section of the hydrogel was lyophilized utilizing the cryofracture method. The lyophilized sample was attached to a coverslip via a coated thin film of chromium and carbon. The coating also worked to prevent the accumulation of static electric charge on the surface during electron irradiation and to avoid scanning faults and other image artifacts. The samples were sprayed with colloidal gold particles and then left drying at room temperature (25 °C) before scanning.

3. Results and discussion

3.1. Hydrogel characterization

In order to obtain an optimized hydrogel by mixing the galactomannan from C. grandis seeds and κ-carrageenan, a full factorial design² was performed (see Supplementary material). According to the results, it was possible to obtain a stable hydrogel composed by 1.7% (w/v) of galactomannan and 0.5% (w/v) of κ-carrageenan, with 0.2 M of CaCl₂ at pH 5.0 which was capable of supporting higher temperature (40.6 °C).

Fig. 1. One-dimensional 1H NMR spectra (4.6–5.3 ppm region) of the purified galactomannan, κ-carrageenan and of hydrogel obtained by the mixture of these two polysaccharides at 45 °C (A). The impact of temperature variation was observed by the broken horizontal lines on the 1H chemical shifts observed in the spectra of purified galactomannan (B), κ-carrageenan (C) and the hydrogel (D).

3.1.1. Nuclear magnetic resonance (NMR) spectroscopy

We characterized the hydrogel formed by κ-carrageenan and galactomannan using 1D 1H NMR spectroscopy. The 1H chemical shifts of these two polysaccharides are similar irrespective the spectra were run using purified polysaccharide solutions or hydrogel. Fig. 1 exemplifies this observation for the 4.6–5.3 ppm region of the spectra run at 45 °C. In this region, we can easily identify the anomeric protons of the α-galactopyranosyl (α-G1) and β-mannopyranosyl (β-M1) units at 5.11 and 4.84 ppm, respectively, of the galactomannan (bottom spectrum in Fig. 1A). It is also possible to visualize the anomeric protons of the 3,6-anhydro-α-galactose (α-K1) and of the β-galactopyranosyl unit (β-K1) at 5.19 and 4.72 ppm, respectively, of the κ-carrageenan (middle spectrum at 45 °C in Fig. 1A). We can also identify H4 of the 4-sulfated β-galactose (β-K4) due to the characteristic downfield shift of the 4-sulfation site. These results are in accordance with the
solution NMR analysis of galactomannans and carrageenans previously reported in literature (Albuquerque et al., 2014; Cheng et al., 2013; Yao, Wu, Zhang, & Du, 2014; Farias et al., 2008; Bosco, Segre, Mierutus, Cesa’ro, & Paololetti, 2005).

These characteristic $^1$H NMR signals of the galactomannan and κ-carrageenan show no significant shifts in the $^1$H NMR spectrum of the hydrogel (top spectrum in Fig. 1A), except for a very modest ~0.02 ppm upfield shift of β-K4. Clearly this is a consequence of interaction of 4-sulfate groups with calcium ions in the hydrogel formulation. These results indicate that κ-carrageenan and galactomannan form a physical type hydrogel, containing semi-interpenetrating polymer network (SEMI-IPN). Clearly, these polysaccharides did not form a chemical type hydrogel, which would result in shifts of their $^1$H-signals.

We also investigated the impact of temperature on the $^1$H spectra of the purified galactomannan (Fig. 1B) and κ-carrageenan (Fig. 1C) and of hydrogel (Fig. 1D). The $^1$H signals show marked downfield shifts as the temperature increases from 25 °C to 55 °C (shifts indicated by horizontal broken lines in the Panels). However, more significant, the shifts are similar on the spectra run with the purified polysaccharides solutions (Fig. 1B and C) or with the hydrogel (Fig. 1D). Some $^1$H signals are broad or even not identified at 25 °C, especially those of the β-anomers, mostly because of the interference of HOD signal at this temperature and of the water suppression by presaturation. This is particularly relevant for the $^1$H spectra of the hydrogel at 25 °C and 35 °C (bottom panels in Fig. 1D), which suggest strong intermolecular interaction and self-organization of the polysaccharides in the gel state. The $^1$H signals were recovered as the temperature increases, indicating disruption of the self-organization due to the increased dynamics of the polysaccharides solutions. This is a typical phenomenon of viscous solutions obtained with macromolecules (Shapiro, 2011).

### 3.1.2. Stability and shelf life evaluation of the hydrogel

The pH is an important parameter for evaluation of stability and biocompatibility of hydrogel. The hydrogel formed using galactomannan and κ-carrageenan showed an average pH = 5.70, without significant variations (p < 0.05). According to Segura, Camargo Junior, Bagatin, and Campós (2010), this pH range is considered suitable for various applications, especially in cosmetics, food and pharmaceutical industries. According to the microbiological analysis, there was no observed microorganism growth in any of the experimentation days (data not shown). The colorimetric results for the $L^*$ and Y parameters are shown in Table 1. $L^*$ denotes the light content into the sample. The opacity (Y%) is another important characteristic that indicates the capacity of biomaterials to act as a barrier against light, and it is also a way to relate the major or minor degree of miscibility of polymers (Li & Shimizu, 2006).

The experimental results have shown a significant increase in luminosity of approximately 24% along the course of the measurements followed by a reduction of opacity of 29%. On the first days it is most likely that the hydrogel is on a globular/double helix stage which scatters light more efficiently. With the aging of the hydrogel, as it keeps reacting with the free calcium ions, these helices start aggregating and organizing in junction zones in a way that reduces scattering and turns it more transparent. This comportment was also observed by the changes in opacity, which exhibited a reduction until the 30th day, with a small increase at the final of the experimental time (60th and 90th days). This confirmed the reorganization of the polysaccharide chains of the hydrogel that is reflected in a less opaque macroscopic sample, it is, more transparent. A similar observation was reported by Silva, Pereira, Carvalho, and Ferrua (2007), who showed that starch gels suffer structural changes along its formation with decreasing opacity.

### 3.1.3. Rheometry

The hydrogel rheological studies are depicted on Fig. 2 Fig. 2A shows an oscillatory experiment where the elastic (storage), $G'$, and dissipation (loss), $G''$, moduli are monitored as a function of the oscillatory frequency. Regarding hydrogels, once the crosslinks between the different chains of the polymers are established, the network thus obtained shows a solid viscoelastic behavior and, sometimes, a pure elastic behavior (Hennink & van Nostrum, 2002; Cuggino, 2008). This can be visualized in Fig. 2A, which shows $G' > G''$ as a function of the oscillatory frequency. For longer periods of shelf storage, the gel matrix is reinforced and, in addition to $G' > G''$, the values of individual moduli do not vary substantially with frequency. These characteristics are a pristine signature of gel rheological behavior. Visually the hydrogel is self-standing (see Supplementary material). For short shelf storage periods, although $G' > G''$ and the gel behavior still preserved, the data indicate that the long-range network structure is not fully developed. For instance, at higher frequencies, both storage and loss moduli increase indicating the presence of a small length scale structure. In Fig. 2A the elastic modulus $G'$ increases too steeply in contrast to what is expected for continuous gels. Instead its behavior resembles that of a packing of emulsion droplets at the jamming transition. It is important to note the classical gel behavior (parallel $G'$ to $G''$ as a function of frequency) occurs above the 15 days storage period. In addition, the gel formed can be considered a strong gel when compared to the pure galactomannan gel (Albuquerque et al., 2014). Therefore, during the shelf storage (4°C storage temperature) period the gel long-range structure is consolidated. These results were similar to those obtained by Pinheiro et al. (2011), who reported long-range gellation for mixtures of κ-carrageenan and galactomannan extracted from Gleditsia triacanthos seeds (60/40, respectively) and Sophora japonica (60/40, respectively).

Macrosopic hydrogels can support only a limited amount of stress and break when stress or strain exceeds some yielding limit. The data show the two main characteristics of the formed gel: a linear elastic region where $G'$ is constant regardless of the stress applied; and a yield stress where the gel structure is destroyed and the system starts flowing (Fig. 2B). Although $G'$ and $G''$ lose meaning at nonlinear regions, we will consider the point at the cross over, when $G' = G''$, as the stress necessary to yield. Conversely $G''$ does not show a linear region. Instead there is a weak overshoot at the crossover point. This overshoot does not exist in pure galactomannan samples from C. grandis (Albuquerque et al., 2014) and is most likely due to the existence of long chains in the κ-carrageenan as also found in xanthan gum (Hyum, Kim, Ahn, & Lee, 2002). During a stress test, in order to keep oscillating frequency fixed, as the stress (strain) increases the shear rate has to increase. Slow structure relaxation depends on the applied shear rate and gets faster as the shear rate increases. This explains the increase in dissipation $G''$ as the stress increases. However there is a peak at the point where the shear rate reaches the oscillating frequency causing dissipation to become inefficient. Overall, the increase in loss, $G''$, or storage, $G'$,
Fig. 2. Rheological measurements of the best hydrogel formulation obtained from the full factorial design $2^3$. A—Elastic ($G'$) and viscous ($G''$) moduli as a function of the angular frequency, dynamic oscillatory flow. B—Elastic ($G'$) and viscous ($G''$) moduli as a function of the shear stress, dynamic oscillatory flow. C—Elastic ($G'$) and viscous ($G''$) moduli as a function of the storage time (90 days). D—Stress at crossover as a function of the storage time (90 days).

Fig. 3. SEM micrographs of the surface and matrix of the galactomannan/κ-carrageenan hydrogel at the first day (A and B, respectively) and after 90 days-period of storage at 4 °C (C and D, respectively).
moduli as a function of the storage time (Fig. 2C) corroborates what was previously discussed about an increase in the organization of the polysaccharide chains in the hydrogel matrix.

The elastic modulus for day 1 is very similar to the G′ for pure galactomannan. It is worth noting that the galactomannan/κ-carrageenan combination is stiffer – higher G′ – but less tough, i.e. the area G′ x stress is smaller than that of the pure galactomannan (Albuquerque et al., 2014). The accommodation of the interstitial entangled galactomannan network reduces the elastic modulus G′ – there is a lower energy required to deform the network – at the same time that it increases the dissipation via the loss modulus locally. Finally, as the shelf storage period increases, there was an increase in the stress where the crossover between elastic and viscous moduli occurred (Fig. 2D). These results corroborate those presented in Fig. 2A, indicating that the hydrogel matrix was getting more organized and stiffer along the shelf storage period.

3.2. Scanning electron microscopy (SEM)

Studies of scanning electron microscopy were carried out to characterize the microstructure morphology of the freeze-dried galactomannan/κ-carrageenan hydrogel. Fig. 3 shows the SEM images of the surface and matrix of the hydrogel at the 1st day after preparation and after 90 days of storage at 4 °C. In the 1st day, the surface image indicates a rough and wavy morphology (Fig. 3A). In contrast, the matrix shows a 3D network, composed of 2D folded sheets, allowing for a reasonable interconnection between macropores (Fig. 3B). The roughness on the surface of the hydrogel is attributed to the presence of galactomannan (Lombardi & Mercê, 2003). It can also be observed an interconnection between pores that could be assigned to the crosslinking network formation between the sulfate group from κ-carrageenan chains and Ca2+ ions in the hydrogel formulation. Pore connectivity determines the easy flow of drugs and indicates if the hydrogel is appropriate for tissue engineering applications (Varghese, Chellappa, & Fathima, 2014).

After 90 days of storage at 4 °C, both surface and matrix of the hydrogel changed (Fig. 3C and D). The surface of hydrogel became less rough and the pores in the matrix became larger than the first day. This could be attributed to the organization of the polysaccharide chains in thin helices that starts to aggregate, thus reducing the fibers, and increasing the pores of the hydrogel matrix. Besides the fact that the porosity created by cryofracture method does not represent the hydrated porosity since the lyophilization process creates artifacts, the SEM analysis of the hydrogel remains interesting since these artifacts are linked to the real structures and the density of the hydrogels. The same observation was reported for thermosensitive chitosan hydrogels (Assaad, Maire, & Lerouge, 2015). The differences observed along the 90 days-period suggests that the hydrogel morphology can be modified by the aging time and depending on the targeted application, a smaller or larger porosity may be more favorable, given that porosity influences the drug release and cell invasion in tissue regeneration applications.

4. Conclusion

According to the results obtained of a full factorial design based on rheological parameters, it was possible to obtain a stable hydrogel composed by 1.7% (w/v) of galactomannan (from C. grandis seeds) and 0.5% (w/v) of κ-carrageenan, with 0.2 M of CaCl2 at pH 5.0. This hydrogel showed a temperature resistance until 42 °C without losing its shape. Furthermore, there were no significant changes in pH and no microbial contamination. Nevertheless it was observed a decrease in opacity and an increase in the transparency along in the 90 days-period of storage at 4 °C. The nuclear magnetic resonance has indicated the absence of covalent bonds which classify the investigated hydrogel as a physical gel, and confirmed that the temperature plays an important role in the maintenance of this physical gel matrix stability. Furthermore, the thermo-rheological tests confirmed a higher organization of the polysaccharides in the hydrogel matrix over the storage period with the elastic modulus always higher than the viscous modulus. The SEM analysis of the hydrogel matrix showed interconnected and macropore architecture with a rough surface. These pore matrix interconnections may lead to an easy flow for biomolecules as well as scaffold matrices for tissue engineering. These results showed that this hydrogel has good and stable physical properties with potential application in medical and cosmetic industries.

Acknowledgments

P.A.G.S. is a recipient of a scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the author P.B.S.A. is a recipient of a scholarship from the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), M.G.C.C., M.T.S.C. and P.A.S.M. express their gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research grants and fellowship. The authors acknowledge Centro de Tecnologias Estratégicas do Nordeste (CETENE) and Centro Nacional de Ressonância Magnética Nuclear by analytical support and CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by financial support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.carbpol.2015.08.042.

References


