

Nanometric organisation in blends of gellan/xyloglucan hydrogels



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ABSTRACT

Mixtures of gellan gum (GL) and a xyloglucan (XGJ) extracted from *Hymenaea courbaril* seeds were prepared in a solution of 0.15 mol L⁻¹ NaCl. Rheology measurements revealed that 2.4 g L⁻¹ pure GL formed a brittle hydrogel, and GL-XGJ blends showed improved pseudoplastic character with higher XGJ contents. SAXS analyses showed that the R_g dimensions ranged from 1.3 to 4.9 nm, with larger values occurring as the amount of XGJ increased, and diffusion tests indicated that better diffusion of methylene blue dye was obtained in the network with a higher XGJ content. AFM topographic images of the films deposited onto mica revealed fewer heterogeneous surfaces with increased XGJ contents. The water contact angle revealed more hydrophobic character on all of the films, and the wettability decreased with increasing amounts of XGJ. Therefore, the demonstrated benefit of using XGJ blends is the production of a soft material with improved interface properties.

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1. Introduction

The development of polysaccharide-based matrices, including hydrogel-based filters (Lieleg & Ribbeck, 2011) and scaffold materials for three-dimensional cell cultures (Jayawarna et al., 2006) and tissue engineering (Drury & Mooney, 2003), can be improved by promoting interactions between various polysaccharides. This strategy yields improved properties for different biotechnological applications because many polysaccharides and their derivatives have the ability to form hydrogels (Delair, 2012; Jó, Valenga, Petri, Lucyszyn, & Sierakowski, 2009; Pakulska, Ballios, & Shoichet, 2012). To design a suitable matrix, the mechanism of the hydrogel network formation, which affects the pore size, surface area and the ability of the formed hydrogels to retain water, must be considered in addition to the chemical nature of the constituents. According to Frampton, Hynd, Shuler, & Shain (2011), the hydrogel must also be able to provide attachment sites for anchorage-dependent cell types. In addition, previous studies reported the importance of

evaluating the diffusibility on the gel network, involving properties such as mass transfer, limiting concentration of the bulk substrate, bulk buffer level and bulk pH (Kuhn, Peretti, & Ollis, 1993; Zhang & Furusaki, 2001).

Hydrogels based on xyloglucan (XG) from different origins have been previously studied alone and in mixtures with other hydrocolloids. For example, Lima-Nishimura et al. (2003) developed XG-agar-based hydrogels for use in plant tissue cultures. In other studies, authors demonstrated that the degalactosylation of xyloglucan generated a thermoreversible hydrogel (Busato, Reicher, Domingues, & Silveira, 2009; Freitas, Busato, Mitchell, & Silveira, 2011). The XG can be extracted from leguminous seeds, where they have a storage function (Reid, 1985). The XG macromolecular structure is formed by a 1,4- β -D-glucopyranosyl backbone partly substituted by 1,6- α -D-xylopyranosyl side chains, which are in certain cases, further substituted by a 1,2- α -D-galactopyranosyl residue.

Commercial applications most commonly use XG extracted from *Tamarindus indica* seeds, although other plant sources have been proposed in the literature. Important commercial applications of XG in the food, pharmaceutical and medical industries have been demonstrated (Itoh et al., 2008; Maeda, Yamashita, & Morita, 2007). Our research group has extensively studied a xyloglucan extracted

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from *Hymenaea courbaril* seeds (Caesalpinoideae) and its derivatives (Freitas et al., 2005; Jó et al., 2009; Lima-Nishimura et al., 2003; Lucyszyn et al., 2009; Ribeiro, Arizaga, Wypych, & Sierakowski, 2009; Sierakowski, Castro, Lucyszyn, & Petri, 2007). The seeds are commonly referred to as jatobá or jataí and are found in Brazil from the Piauí to the north of the Paraná States (Lorenzi, 2002). Every mature tree (20 years old) typically produces 10 kg of seeds per year from which approximately 4.5 kg of raw xyloglucan can be obtained (Lima-Nishimura, Rechia, Ganter, Reicheir, & Sierakowski, 1995).

Freitas et al. (2005) investigated the use of endocellulases from several *H. courbaril* xyloglucan sources and compared those with XG from *T. indica*. The results showed structural and conformational differences between these sources, with the molecule from *H. courbaril* displaying a more rigid structure than the XG extracted from the *T. indica* source. This variation in the oligosaccharides can influence the adsorption of XG on the substrates, as observed by Sierakowski et al. (2007), where the XG from *H. courbaril* generated thicker films on Si/SiO₂ than XG from other sources. Using computer simulations, Zhang, Brumer, Ågren, and Tu (2011) also observed the effect of different XG oligosaccharides on the adsorption on the cellulose surface, which requires a more rigid conformation for a more favourable adsorption.

Gellan gum (GL) is a bacterial polysaccharide obtained from *Sphingomonas elodea*, and its glycosidic backbone is a repeating tetrasaccharide unit (-3)-β-D-Glcp-[1-4]-β-GlcpA-[1-4]-β-D-Glcp-[1-4]-α-L-Rhap-[1] with one L-glycerate per repeating unit and one acetate occurring every two sequences as esterified substituents (Jansson, Lindberg, & Sandford, 1983; Milas & Rinaudo, 1996; Miyoshi, Takaya, & Nishinari, 1996; Morris, Nishinari, & Rinaudo, 2012). Removal of these substituents by de-esterification and the proper choice of salt-polymer formulation improve the gelling capacity (Funami et al., 2009; Noda et al., 2008). The addition of a small amount of salt readily promotes a conformational transition into an ordered double-helix chain, promoting the formation of a strong and rigid gel (Miyoshi et al., 1996; Nitta & Nishinari, 2005; Rinaudo, 2008). GL is approved by the US FDA and the European Union (E 415) for use in food products and for use as a substrate in tissue engineering applications (Amin & Panhuis, 2011; Smith, Shelton, Perrie, & Harris, 2007). In the food industry, GL functions as a stability enhancer, a consistency agent and a flavour releaser (Rodríguez-Hernández, Garnier, Tecante, & Doublier, 2003). In biotechnology, the versatility of GL solutions has been used to develop drug delivery systems for nasal and ophthalmic formulations by the formation of *in situ* gel systems under physiological conditions (Carlfors, Edsman, Petersson, & Jörnving, 1998; Matricardi, Cencetti, Ria, Alhaique, & Coviello, 2009). Recently, GL hydrogel systems have been studied as new substrate materials for human and vegetable tissue engineering applications (Oliveira et al., 2010). In addition, GL hydrogel systems may possess great potential for the development of biofilms for medical applications (Sutherland, 2001).

According to our literature search, the description of the structural network formation of hydrogels based on xyloglucan and gellan gum has not been fully investigated. Knowledge about the synergistic interactions of xyloglucan is of commercial interest due to the prospect of generating novel functionalities, primarily related to its rheological and textural characteristics. In addition, the cost of traditional products can be reduced by decreasing the gellan content in systems with equivalent or improved properties that may be derived from new sources of xyloglucan (Milas & Rinaudo, 1996).

In an effort to contribute to the synergistic studies between GL and XG, we present evidence of the nanometric organisation of mixtures between freshly extracted xyloglucan from *H. courbaril* (XGJ) seeds and gellan gum (GL) in a saline environment.

The hydrogels formed upon mixing both polysaccharides in varied proportions were evaluated by rheology, differential scanning micro-calorimetry (μ-DSC) and small angle X-ray scattering (SAXS). The nanoscale organisation of the corresponding films was investigated by atomic force microscopy (AFM), and the surface properties were evaluated by contact angle (CA) measurements. In addition, diffusion tests were performed using methylene blue (MB) as a hydrophobic probe to evaluate the possible use of the hydrogels for biotechnological purposes.

2. Materials and methods

Gellan gum (GL) was purchased from Sigma-Aldrich (lot no. BCB7653V) and used without further purification. Using atomic adsorption, the salt content (% w/w) of the GL was measured to be 2.10, 0.74, 0.26, and 0.05 for K, Na, Ca, and Mg, respectively. The Blumenkrantz method (Blumenkrantz & Asboe-Hansen, 1973) revealed the uronic acid content (u.a.%) of the GL to be 29.4 ± 1.1 u.a.%.

Xyloglucan (XG) was extracted from *H. courbaril* seeds that were harvested in 1999 from trees growing in Cuiabá City in Mato Grosso State, Brazil. The isolation of crude XG was performed via aqueous extraction (Freitas et al., 2005). The crude XG was purified by solubilisation in distilled water at 30 °C, sonication for 30 min using a solid probe of 25 mm with a 40% amplitude (15 W) (SONICS VCX 750) and separation of the insoluble fraction by centrifugation at 6311 × g. The soluble fraction was precipitated in commercial ethanol (98%, Divalcool Ltda, Brazil), washed with acetone (Sigma Aldrich®) and oven dried at 50 °C to yield the purified xyloglucan (XGJ). Using size exclusion chromatography (GPC/SEC), the weight-average molar mass was determined to be 447.50 kg mol⁻¹, with a polydispersity index of 1.66 ± 0.07. Additional information on the compositions (Table S1 and Fig. S1) and cytotoxicity assay (Fig. S2) of XGJ are shown in the Supplementary data.

2.1. Preparation of GL/XGJ dispersions

GL and XGJ were separately dispersed in 0.15 mol L⁻¹ NaCl to a final concentration of 2.4 g L⁻¹. Before mixing, the dispersions were pretreated as follows. To reach complete solubilisation, the GL solution was heated to boiling in a sealed vessel for approximately 20 s in a microwave system (900 W). In this process, the water loss was considered to be negligible. The XGJ solution was stirred overnight at 23 ± 2 °C. The solutions were mixed at various volume ratios of GL/XGJ to produce a final concentration of 2.4 g L⁻¹.

2.2. Rheological measurements

The flow behaviour of the systems was analysed using a Rheostress1 (Haake, Karlsruhe, Germany) plate-plate rheometer with a PP20 sensor (with a diameter of 20 mm and a gap of 1 mm). The flow behaviour was monitored in a controlled shear stress mode at 25 ± 0.1 °C. The measurements were performed at low shear rates (0.01 to 100 s⁻¹), and the results were mathematically analysed using the Ostwald-de-Waele (Power-law) model, which is expressed in the following equation:

$$\tau = k \times \dot{\gamma}^n \quad (1)$$

where τ is the shear stress (Pa s⁻¹), K is the flow consistency index (Pa s⁻¹), $\dot{\gamma}$ is the shear rate (s⁻¹), and n is flow behaviour index (dimensionless), where $n = 1$ corresponds to an ideal Newtonian fluid, and values of $n < 1$ reflect a pseudoplastic property of the fluid (Rao, 2007). The software RheoWin 3 Data Manager (Haake, Karlsruhe, Germany) was used to obtain the rheological results, the Origin 8.5 software was employed to obtain the mathematical

parameters, and the best theoretical fit curves were based on the chi-squared (χ^2) results.

2.3. Differential scanning micro-calorimetry analysis (μ -DSC)

The μ -DSC analyses were performed using a micro-DSC-III calorimeter (Setaram, Caluire, France). Approximately 500 mg of the fresh solution was placed into a DSC pan and hermetically sealed. An equivalent amount of the 0.15 mol L⁻¹ NaCl solution was used as the reference. The process of heating and cooling was performed in temperature cycles from 5 to 110 °C at 0.5 °C min⁻¹. The experiments were performed in duplicate.

2.4. Small angle X-ray scattering (SAXS) measurements

The SAXS experiments were performed on the D01B-SAXS1 beamline of the LNLS (Brazilian Synchrotron Laboratory, Campinas, SP, Brazil). The scattered beam ($\lambda = 0.1488$ nm) was detected using a Pilatus 300k area detector placed 1589.170 mm away from the sample, resulting in a scattering vector range ($q = (4\pi/\lambda) \times \sin \theta$, where 2θ = scattering angle) from 0.07 to 2.25 nm⁻¹. The solutions were transferred to a sample stage with the mica windows placed perpendicular to the X-ray beam (Cavalcanti et al., 2004). Silver behenate powder was used as a standard to calibrate the sample-to-detector distance, the detector tilt and the incident beam position. Transmission, dark current, mica and solvent corrections were performed on the 2D image before further data processing. The isotropic scattering patterns were radially averaged. All of the measurements were performed at room temperature (25 °C).

SAXS data analysis was performed using the Irena evaluation routine implemented in the commercially available Igor Pro Software (WaveMetrics, Portland, USA) (Ilavsky & Jemian, 2009; Kline, 2006). A multi-level unified fit was used to describe the two levels of structural organisation evident in the scattering data (Beaucage, 1995, 1996). In this method, the scattering provided by each structural level is the sum of a Guinier exponential-form and a structurally limited Power-law tail. A generalised equation, representing any number of spherical levels, is expressed as follows:

$$I(q) = \sum_{i=1}^n G_i \exp\left(\frac{-q^2 R_{gi}^2}{3}\right) + B_i \exp\left(\frac{-q^2 R_{g(i+1)}^2}{3}\right) \times \left[\frac{(\text{erf}(q R_{gi}/\sqrt{6}))}{q}\right]^{pi} \quad (2)$$

where n is the number of structural levels observed, G is the Guinier prefactor, R_g is the radius of gyration, and B is a prefactor specific to the Power-law scattering, which is specified as the decay of the exponent P (Beaucage, 1995, 1996).

2.5. Diffusion tests

Diffusion of the methylene blue (MB) dye solution (0.5 g L⁻¹, in water) through never-dried systems was performed according to the literature (Lucyszyn, Quoirin, Anjos, & Sierakowski, 2005). In summary, a 50 μ L aliquot of the probe solution was added to a conic graduated tube filled with 10 mL of sample. The diffusion was analysed by visual observation of the colouring on the scale tube after 24, 48, 96 and 120 h at 25 °C. Each measurement represented three replicates, and the results were expressed as the mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) and Tukey's test with a 95% confidence interval ($P < 0.05$) were performed.

2.6. AFM measurements

Freshly cleaved mica was dipped into the newly prepared mixed polysaccharide systems. Samples were allowed to dry slowly under a controlled temperature (24 °C) and humidity (approximately 45%) to form macroscopically homogeneous films that were stored under the same conditions prior to AFM analysis. The topographic-AFM images of the GL/XGJ films were obtained using a SPM-9500J3 microscope (Shimadzu, Kyoto, Japan) operating under ambient conditions. The images of the films were acquired in the dynamic tapping mode (TM-AFM) with an oxide-sharpened micro-fabricated silicon μ -Masch cantilever, with a nominal spring constant of 4.7 N m⁻¹ and a tip radius of curvature less than 10 nm. The scanning rate was 1 Hz, and 256 $\times$ 256 image data points were obtained. All of the images were flattened and noise-filtered when necessary. The operating point was adjusted to minimise the interaction between the tip and the sample and to avoid soft-layer deformation. The image adjustments were performed using Shimadzu SPM software version 2.4.

2.7. Contact angle (CA) analyses

The GL/XGJ films were submitted to CA analyses in a DataPhysics GmbH (Filderstadt, Germany) tensiometer, model OCA 15 plus. Measurements were performed at 25 °C using a 500 μ L syringe and a needle of internal and external diameters of 1.37 and 1.65, respectively, and a length of 38.1 mm. The hydrophobic/hydrophilic character of the studied GL/XGJ systems was investigated using the sessile drop method, and ultrapure water ($\gamma_l = 72.8$ mN m⁻¹) was used as the test liquid. To calculate the surface free energy (SFE), the ACS20 software from DataPhysics (Filderstadt, Germany) was used. Neumann's method (Li & Neumann, 1992a,b) was employed in which a single contact angle (θ) and the surface tension of the test liquid (γ_l) were used to calculate the total SFE of the deposited films on mica (γ_s) according to the following equation:

$$\cos \theta = 2 \sqrt{\frac{\gamma_s}{\gamma_l}} \times e^{-\beta(\gamma_l - \gamma_s)^2} - 1 \quad (3)$$

The value of β was taken as 0.0001247 (m² mN⁻¹ m⁻¹)². The results were obtained by averaging a minimum of three measurements.

3. Results

3.1. Rheological measurements

The results of the mathematical evaluation of the GL/XGJ systems in 0.15 mol L⁻¹ NaCl are presented in Table 1. The flow curves and images of freshly prepared hydrogel solutions at different GL/XGJ contents are shown in Fig. 1. Solutions with a lower GL concentration exhibited less mechanical resistance than the isolated GL solution, indicating that the presence of XGJ decreased the formation of a GL network in the mixed system.

In the present study, shear rates from 0.05 to 100 s⁻¹ were considered in the mathematical evaluation. The Ostwald–De-Waele model (Eq. (1)) was applied to all of the mixed systems and to pure XGJ. Applying the mathematical adjustment based on the χ^2 value (Table 1), a better fit was obtained with increasing XGJ content in the system. The applied model fits better to pseudoplastic samples; therefore, it was not applied to the pure gellan curve.

In theory, under a shear force, macromolecules are oriented towards the shear direction, and, therefore, the amount of entangled molecules is reduced (K decreases), lowering the flow resistance, which leads to shear-thinning behaviour (Mezger, 2002). These results indicate that an initial shear stress is required

Table 1Rheological parameters and micro-calorimetric results of GL/XGJ blends in 0.15 mol L⁻¹ NaCl solution.

System GL/XGJ (g L ⁻¹)	Parameters ^a			Enthalpy (J g ⁻¹)	
	n^a	K^a (Pa s ⁿ)	χ^2	Exo	Endo
2.4/0.0	n.a.	n.a.	n.a.	-0.033	0.031
1.6/0.8	0.18 ± 0.04	30.63 ± 1.26	31.20	-0.017	0.013
1.4/1.0	0.18 ± 0.01	27.69 ± 1.01	26.03	-0.017	0.013
1.2/1.2	0.24 ± 0.01	13.28 ± 0.45	2.65	-0.014	0.012
0.8/1.6	0.37 ± 0.01	4.10 ± 0.07	0.05	n.o.	n.o.
0.0/2.4	0.40 ± 0.02	2.35 ± 0.15	0.17	n.o.	n.o.

^a n = Flow behaviour index (dimensionless) and K = consistency coefficient parameters obtained by fitting curves using Ostwald–De-Waele model ($\tau = k \times \dot{\gamma}^n$); the values are means of three measurements and $\pm$ standard deviation; n.a.: not applicable; n.o.: not observed.

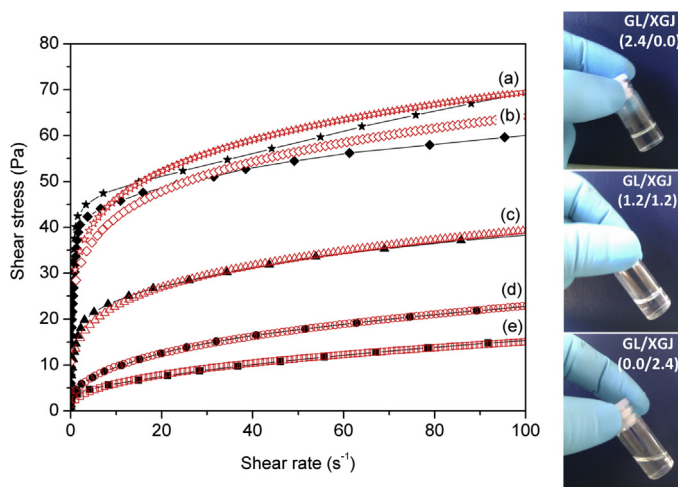


Fig. 1. Flow curves (close symbols) and mathematical approach by Ostwald–De-Waele of the GL/XGJ systems (open symbols) in different ratios: (a) 1.6/0.8; (b) 1.4/1.0; (c) 1.2/1.2; (d) 0.8/1.6; and (e) 0.0/2.4 g L⁻¹ and images of freshly prepared hydrogel solutions at different ratios (in g L⁻¹) 0.15 mol L⁻¹ in NaCl solution at 25 ± 1 °C.

to induce flow in all of the mixtures (Fig. 1). The initial shear stress value decreased as the XGJ content in the blends increased and as the gel network was inhibited.

According to the parameters presented in Table 1, with the exception of pure gellan, all of the other systems demonstrated a pseudoplastic behaviour, as indicated by values of n less than 1. A better fit was obtained at GL/XGJ mass ratios of 1.2/1.2 and 0.8/1.6, and pure XGJ corresponded to a higher degree of pseudoplasticity for these samples.

Therefore, shear-thinning is less evident at higher concentrations of GL, as indicated by an increase in the value of K and a decrease in the value of n . The results show that an XGJ solution at this concentration exhibits better pseudoplastic behaviour, as evidenced by a higher n value than that in mixed solutions. This result suggests that the presence of XGJ contributes to the viscous component of the GL/XGJ blend by forming a continuous liquid phase, thereby, decreasing the formation of the continuous network structure of GL (Jampen, Britt, & Tung, 2000; Nitta et al., 2003).

3.2. μ -DSC measurements

The sol–gel transition temperature and the enthalpies of the different systems were measured using μ -DSC. The cooling and heating sweep curves are presented in the supplementary data (Fig. S3). In NaCl solution, pure GL presented a wide transition in the range of approximately 85–90 °C on heating and 40 °C on cooling ramps (helix-to-coil and coil-to-helix conformational changes). This result is typical for GL with low acyl content, as

discussed by Morris et al. (2012). Temperature shifts associated with the XGJ content were not observed using any of the GL/XGJ mass ratios. The enthalpy values (Table 1) decreased with increasing XGJ content in the blends ($\Delta H^{\text{exo}}_{\text{(pure GL)}} = -0.033 \text{ J g}^{-1}$ and $\Delta H^{\text{exo}}_{\text{(1.2/1.2)}} = -0.014 \text{ J g}^{-1}$).

The endothermic and exothermic events were discussed by Morris et al. (2012) for different GL sources and compositions. The peaks are generally assigned to the coil-to-helix (sol–gel) transition of gellan (Morris et al., 2012; Jampen et al., 2000; Nitta et al., 2003). Therefore, in the case of the studied systems, under higher gellan content, the polysaccharides display a more ordered conformation due to the predominant presence of GL. Under these conditions, to change the conformation, a higher energy demand is required, as observed by the enthalpy results. However, the inclusion of XGJ possibly generates disordered regions throughout the GL network, which explains the decrease in the enthalpy values with increasing xyloglucan content, as corroborated by the rheology measurements.

3.3. SAXS measurements

The SAXS technique was used to obtain nanometric details about the GL/XGJ network in the blends. Although all SAXS curves presented similar patterns, meaningful differences were observed from the results. Fig. 2 presents experimental SAXS data (open circles) and their corresponding unified fits for pure GL, XGJ and a mixture of GL and XGJ (others curves are presented in Fig. S4 in the Supplementary data).

In this unified model, every region can have dimensional and/or structural contributions. The radius of gyration (R_g) can be obtained from the shoulder-type Guinier region, and the linear Power-law decay reveals structural details of the analysed system. Two distinct regions (referred to here as low and high- q regions) were observed for all of the samples using the unified fit procedure. In the low- q region (dashed green line), which represents the largest dimensionality of the system in our SAXS experimental window, no shoulder-type Guinier region was observed. This result indicates that the overall dimension of the polymeric-precursor materials are larger than 1000 Å and can only be observed if a smaller q range was reached by the SAXS instrument. However, the q^{-2} Power-law decay (for example, see Fig. 2(a)) provides an indication about the organisation of these systems. As expected, these results indicate that the samples are gel-like materials. In the high- q region (dotted blue line), a Guinier region is observed followed by a Power-law decay. Voids/pores presence can be identified through the shoulder-type Guinier region which allows determining an average R_g value. Among the systems, the R_g values ranged from 1.3 to 4.9 Å (Fig. 2). The Guinier region shifted to smaller values of q by the addition of XGJ into the system, which indicates that the dimension of this structural level was progressively increased by increasing the polymer amount (dotted blue line in Fig. 2).

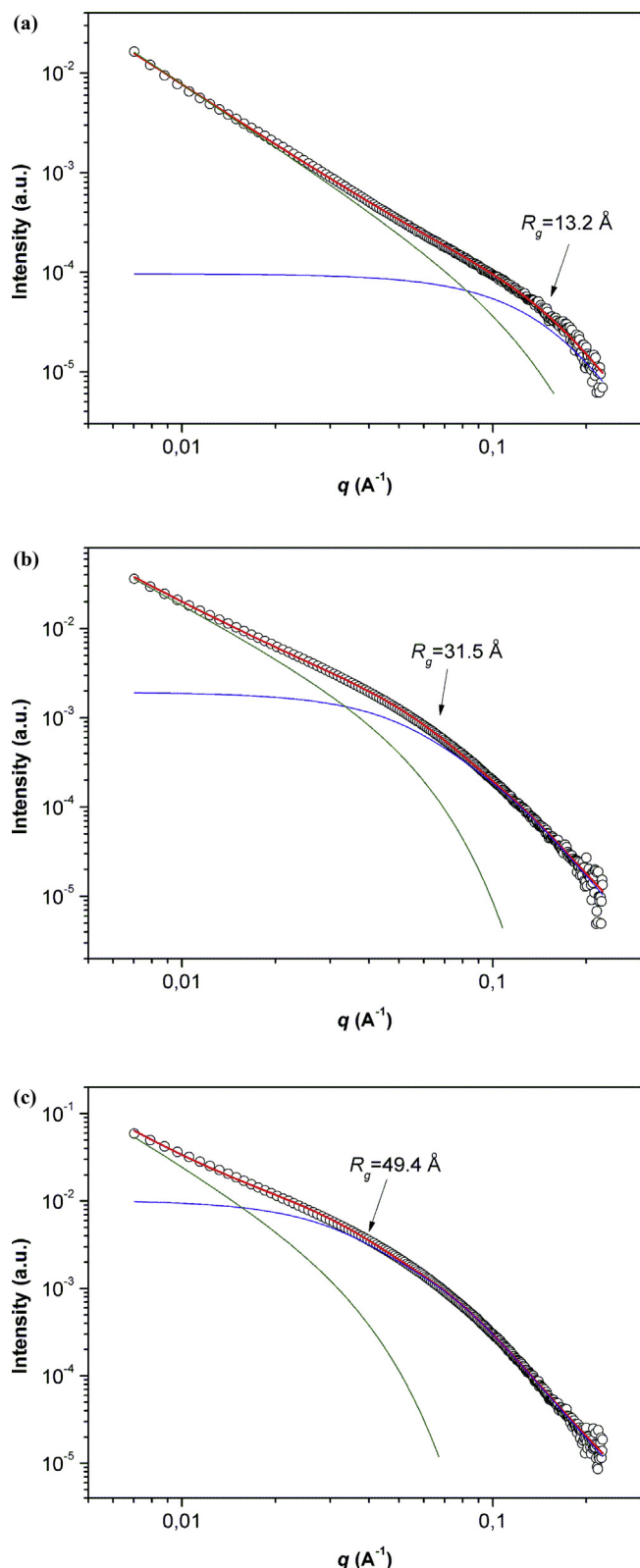


Fig. 2. SAXS profiles of (a) pure GL, (b) 1.2/1.2 g L⁻¹ GL/XGJ mixture and (c) pure XGJ, in 0.15 mol L⁻¹ NaCl at 25 ± 1 °C. The unified fit is shown as a solid red line, and the two fit levels are represented as dashed green and dotted blue lines. (Other GL/XGJ systems are presented in the supplementary data).

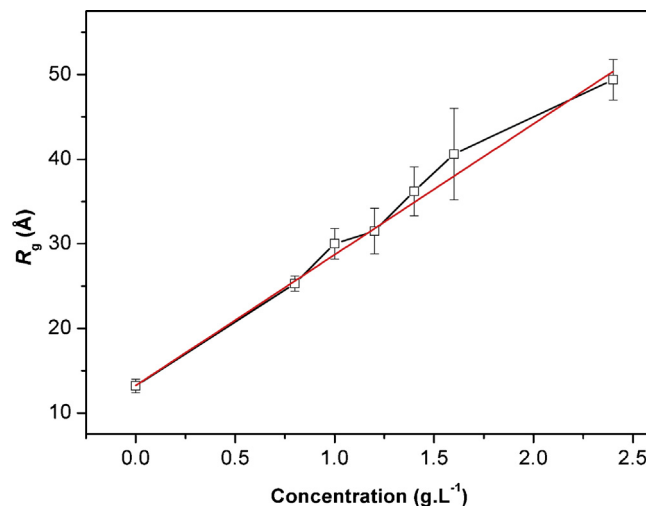


Fig. 3. Dependence of the radius of gyration (R_g) of the voids/pores obtained through the unified fit on the concentration of xyloglucan (XGJ) present in the measured systems.

Fig. 3 shows the dependence of R_g , as determined by SAXS, on the variation of the XGJ concentration used during sample preparation. As a general trend, increasing XGJ concentration increases the R_g value determined in the high- q region. In the isolated GL system (Fig. 2(a)), an R_g value of 13.2 Å is observed.

However, for those solutions containing only XGJ (Fig. 2(c)), the R_g value is 49.4 Å. For GL/XG mixtures, the R_g values were between 13.2 and 49.4 Å. By increasing the amount of XGJ in the mixture, the R_g value increases linearly (as shown by the fit in Fig. 3). The linear fit reinforces the interpretation that describes this structural level as voids/pores whose sizes are influenced by each constituent of the system, depending on its fraction in the system. In this case, the lower limit of the R_g value is related to pure GL, the upper limit corresponds to XGJ and intermediate values are proportional to the fraction between the two polymers. The definition of the R_g value of the voids/pores as a function of the concentration of xyloglucan (XGJ_{conc}) is expressed in the following equation:

$$R_g = 13 + (XGJ_{conc} \ 15.4) \quad (4)$$

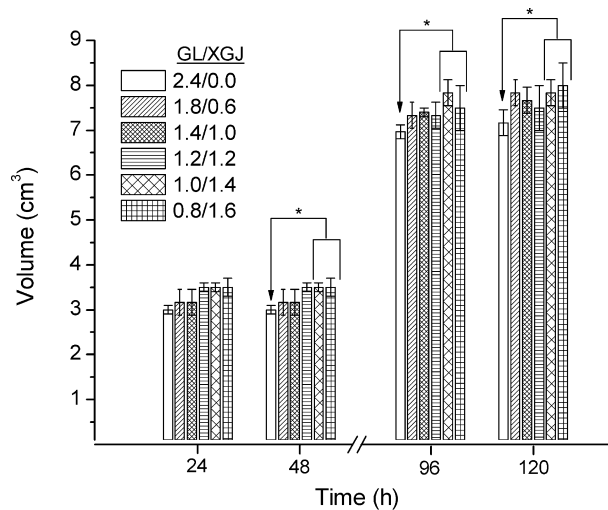


Fig. 4. Diffusion tests of methylene blue dye (0.5 g L⁻¹ in water) in GL/XGJ systems at different volume ratios. (*) indicates a significant difference at $P < 0.05$ with a 95% confidence interval).

3.4. Diffusion tests

Diffusion tests were performed to study the permeability of the systems, and the results are shown in Fig. 4. The diffusion of the MB dye in all of the GL/XGJ mixtures followed the same dependence as a function of time. Significant statistical differences were observed among the pure gellan and the mixed systems in which higher diffusion rates were observed. In this study, the inclusion of XGJ on the GL matrix increased the rate of the dye diffusion in the first 24 h. After 96 h, no statistical significance between the diffusion rates among the samples was observed, as confirmed by ANOVA ($P < 0.05$ 95%) and Tukey's test.

However, an increase in the XGJ content increased the permeation of methylene blue throughout the mixed hydrogel systems compared with the isolated GL. These results are in accordance with a previous study by Ackers and Steere, which showed that separation and diffusion of a solute decreases with the size of the molecule or solute particle and/or the gel strength (Ackers & Steere, 1962). According to Lieleg and Ribbeck, the electrostatic and hydrophilic forces, such as hydrogen bonds and specific

binding interactions, might be used to establish attractive interactions in biological hydrogels (Lieleg & Ribbeck, 2011).

3.5. AFM measurements

The surface morphology of the GL/XGJ systems dried directly onto mica substrates was investigated by AFM, and the images are shown in Fig. 5. The AFM results showed that GL and GL/XGJ blends produce highly aggregated entities compared with isolated XGJ. The series of topographical images showed that the presence of XGJ in saline solution changed the surface morphology after the system was dried. Using the root mean square (rms) values (Fig. 5(f)), we also observed that increasing XGJ concentration caused a decrease in film surface roughness, culminating in a smoother surface aspect in pure XGJ.

3.6. Contact angle (CA) analyses

The CA measurements were obtained to investigate the hydrophilic character of the mixed polysaccharides films. The pure

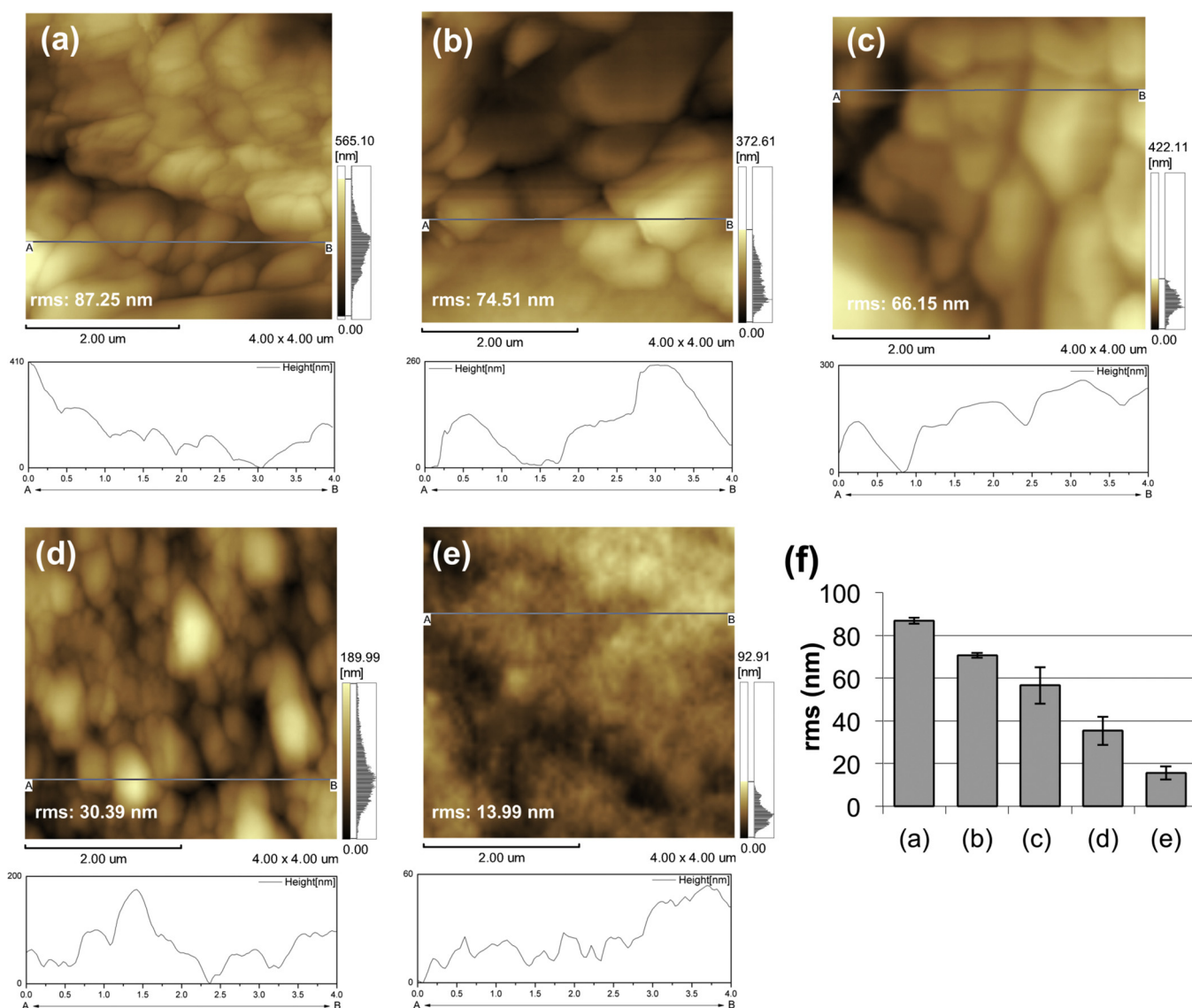


Fig. 5. AFM cross-section topography images of the GL/XGJ systems and the root-mean-square (rms) results obtained for different GL/XGJ volume ratios deposited onto the mica surface (total concentration of 2.4 g L^{-1}): (a) 2.4/0.0; (b) 1.6/0.8; (c) 1.4/1.0; (d) 1.2/1.2; (e) 0.0/2.4 g L^{-1} ; and (f) rms values for each system.

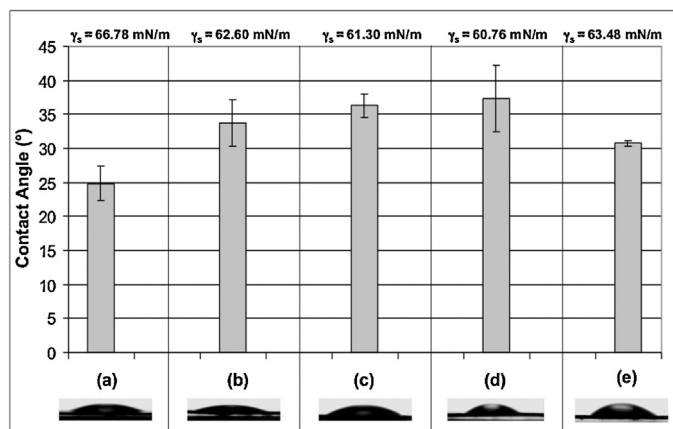


Fig. 6. Contact angle measurements of GL/XGJ films at 2.4 g L^{-1} at different volume ratios on the mica surface: (a) 2.4/0.0; (b) 1.6/0.8; (c) 1.4/1.0; (d) 1.2/1.2 and (e) 0.0/2.4. The surface free energy is represented by γ_s (standard deviation = ± 0.1).

GL and XGJ films showed lower contact angle (CA) values compared with that of mixtures (Fig. 6). These results reveal that the GL films possess a more hydrophilic character at the interface than those of XGJ. These results were expected because GL is negatively charged, whereas XGJ is a neutral polysaccharide.

The value of CA for the mixtures was higher than that of the isolated systems at the same final concentration, which indicates a decrease in the hydrophilic character of the mixed films compared with that of pure GL and XGJ. These results demonstrate the interaction between the biopolymers that leads to a modification of the surface character, most likely by perturbation of the gellan network, changing the chain accommodation of the polymers after the drying process. In addition, xyloglucan presents an amphiphilic behaviour in that the primary chain has a hydrophobic, ribbon-like surface and hydrophilic hydroxyl groups on its sides (Jó, Petri, Beltramini, Lucyszyn, & Sierakowski, 2010; Umemura & Yuguchi, 2005). When GL is included in the system, some association by the hydroxyl groups may occur, resulting in more hydrophobic films, leading the more hydrophobic segments towards the air interface.

Quantitatively, the total surface free energy (SFE) was calculated using Neumann's equation. The results revealed a variation

according to the relative ratio of GL/XGJ. The free energy values increased with the XGJ content, and the CA results indicated the occurrence of different surface arrangements on the mixtures compared with the pure systems, suggesting the possibility of tuning the film surface properties by combining both polysaccharides.

4. Discussion

Under physiological conditions, the content of Na^+ is sufficient to form a strong, brittle and highly hydrophilic gel from gellan alone (León & Rojas, 2007). However, gellan is an expensive material. Therefore, our goal was to develop less hydrophilic hydrogels that demonstrate enhanced diffusion performance for hydrophobic molecules. In addition, hydrogels with enhanced rheological properties allow easier manipulation of the systems. Our strategy consisted of partially substituting the gellan content by another polysaccharide, i.e., a xyloglucan extracted from *H. courbaril* seeds (XGJ). Toxicity tests (in the supplementary data) demonstrated that XGJ exhibits low *in vitro* cytotoxicity, which is desirable for investigations concerning biological and biotechnological applications in foods or pharmaceuticals products.

Our study obtained hydrogels with distinct properties compared with pure gellan gels, revealing the synergistic effect of combining both polysaccharides.

Rheological experiments demonstrated the increased shear-thinning behaviour of the combined systems with the progressive inclusion of XGJ in the GL network, which can be explained by the random coil conformation of XGJ solutions (Huang, Takhar, Tang, & Swanson, 2008; Paulsson, Hägerström, & Edsman, 1999). Therefore, the inclusion of another hydrocolloid, XGJ, as proposed here, is a viable strategy to obtain more flexible systems by inhibiting the formation of a fully interconnected GL network.

Several authors have described the interaction of gellan gum and xyloglucan. Using calorimetric experiments, Ikeda et al. (2004) observed a shift to higher temperatures at the onset of the exothermic peak, corresponding to the coil-to-helix transition of sodium-type GL in the presence of tamarind xyloglucan, using water as the solvent. The authors observed that in the presence of a tamarind xyloglucan concentration of 6.0 g L^{-1} , it was no longer possible to observe the GL (in a concentration of 1.5 g L^{-1}) coil-to-helix transition (Ikeda et al., 2004). In this study, using a

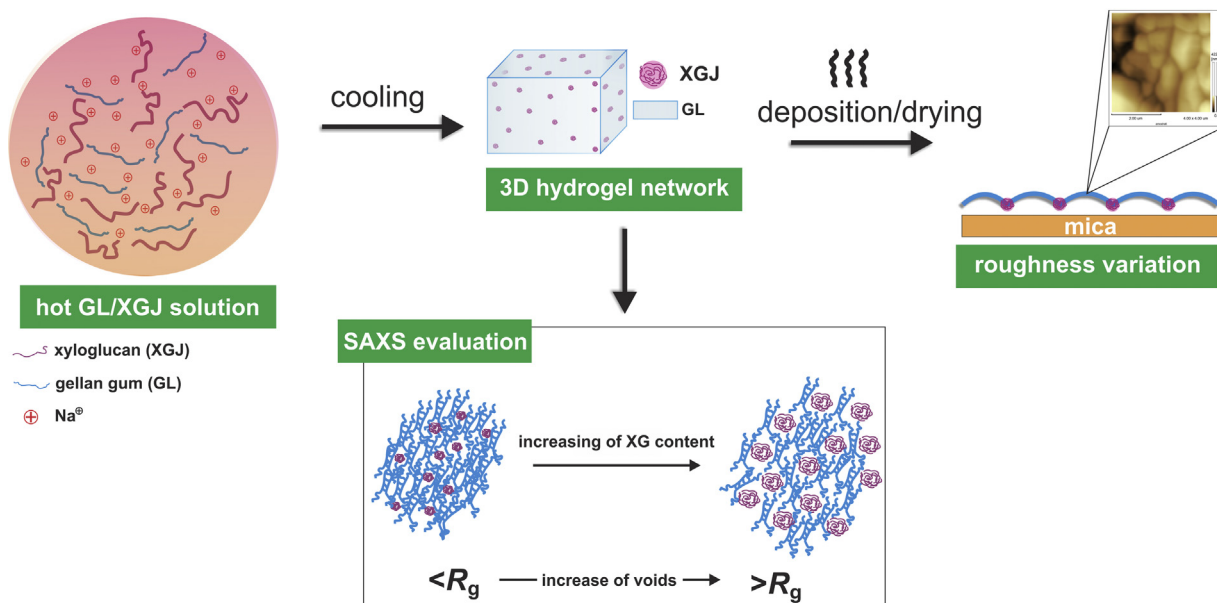


Fig. 7. The idealised structure of the hydrogels (by SAXS) and films (by AFM) based on blends of gellan gum (GL) and xyloglucan (XGJ) hydrocolloids.

deacetylated potassium-type GL in NaCl solution (0.8–2.4 g L⁻¹), the calorimetric experiments showed no evidence of GL and XGJ synergism because the GL coil-to-helix transition was observed in all of the mixed samples at approximately the same temperature.

SAXS analyses clarified certain structural aspects of the freshly prepared GL/XGJ systems. In all of the systems, the characteristic R_g value increased with increasing XGJ content. The R_g values can be interpreted as voids/pores, which favour the diffusion capacity of a hydrophobic probe (MB) in systems with high XGJ content. The possibility of varying the porosity of a mixed system (according to the formulation) can provide certain strategies to entrap molecules or particles and, thereby, control their diffusion behaviour.

Better diffusion of water and other components (such as nutrients in tissue culture) can occur in modified media formulated by freshly prepared GL/XGJ blends. To achieve the best conditions for this application, it is necessary to develop a hydrogel with good stability, avoiding easy mechanical disruption. Thus, the stability is achieved with the inclusion of XGJ at low concentrations, resulting in hydrogels with increased viscoelastic behaviour compared with the isolated GL system.

In addition, gels with increased diffusion properties may be preferable in plant tissue cultures. Better diffusion of nutrients in the tissue culture could occur in modified media formulated using GL/XGJ blends.

AFM and CA analyses were performed in dried systems. Upon drying, certain surface rearrangements of the mixture occur, leading to more hydrophobic domains at the interface, which are related to a rougher surface topology. Low hydrophilic character is a desirable property in edible films. Therefore, the present studied systems could be a promising alternative for this application. The properties observed in the mixed systems can be further exploited in material coatings to prevent the excess of solvated water. For each application, the best selection of polymer matrix to develop a film network and the inclusion of specific additives can provide protective properties for coated products, providing a suitable storage atmosphere (León & Rojas, 2007; Umemura & Yaguchi, 2005).

In summary, through the combination of two polysaccharides, GL and XGJ, the rheological, surface and porous characteristics could be simultaneously varied, producing more flexible hydrophobic hydrogels with higher porosity compared with pure gellan.

Based on the SAXS and AFM results, a schematic drawing of the nanometric organisation of the GL/XGJ systems is proposed on Fig. 7.

5. Conclusions

In this study, we demonstrated the association between gellan gum (GL) and a xyloglucan extracted from *H. courbaril* seeds (XGJ). In NaCl solutions, the GL forms a brittle gel; however, the addition of XGJ altered this characteristic in the GL/XGJ blends. The rheological properties of the mixed systems are dependent on the XGJ content, and the pseudoplastic character of the gels increases with increasing XGJ content. We suggest that XGJ forms a discontinuous phase within the GL gel network, inhibiting full interconnection between the GL chains. The SAXS results revealed the gel organisation on a nanometric scale of the voids/pores (R_g) and indicated that the void sizes increased with increasing XGJ content. The increasing pore sizes were also evidenced by the MB dye diffusion because the porous characteristics of the network produced faster dye diffusion in the presence of XGJ. AFM and contact angle studies, performed on GL/XGJ deposited films, revealed that there were differences in the roughness and hydrophilicity of the dried film surfaces. This information also indicates that the association of both polysaccharides can be used to tune the surface properties of desired substrates.

Therefore, our study demonstrated the benefits of employing XGJ in the preparation of hydrogels in combination with GL. In addition to providing gelling systems with different properties from those of pure gellan, inclusion of XGJ offers the major advantage of reducing the cost by decreasing the content of the gelling agent.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.carbpol.2014.07.070>.

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