



Mechanical and optical characterization of gelled matrices during storage



Gabriel Lorenzo^{a,b,*}, Noemí Zaritzky^{a,b}, Alicia Califano^a

^a Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CONICET—La Plata, Facultad de Ciencias Exactas, UNLP, 47 y 116, La Plata 1900, Argentina

^b Departamento de Ingeniería Química, Facultad de Ingeniería, UNLP, La Plata, Argentina

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ABSTRACT

The effect of composition and storage time on the rheological and optical attributes of multi-component gels containing locust bean gum (LBG), low acyl (LAG) and high acyl (HAG) gellan gums, was determined using three-component mixture design. The generalized Maxwell model was used to fit experimental rheological data. Mechanical and relaxation spectra of gelled systems were determined by the type of gellan gum used, except LBG alone which behaved as a diluted gum dispersion. Storage time dependence of the gels was analyzed using the rubber elasticity theory and to determine changes in network mesh size the equivalent network approach was applied. Destabilization kinetic was obtained from light scattering results; increasing LAG content improved the long-term stability of the matrices. Almost every formulation exhibited an increment in both moduli during the first 10 days remaining practically constant thereafter or until they broke (binary mixtures with LBG); gels with HAG/LBG mixtures were the least stable.

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1. Introduction

The increasing interest on the interactions between biopolymers has largely been driven by commercial requirements. Food and beverage industries are continually looking for solutions or alternatives to reduce production costs and improve the existing products. Moreover, finding applications for new or existing ingredients continues to be at the forefront of their research (Considine et al., 2011). However, fundamental and comprehensive studies are necessary to reinforce these product changes and new ingredient applications. Interactions between two different polymers can be classified as “associative” if they are thermodynamically more favorable than interactions between the individual polymers of each type and “segregative” if they are less favorable. Knowledge of the origin and nature of the interactions involved can often be used to engineer novel structures and physicochemical properties into food systems.

The physicochemical properties of hydrocolloids are very complex and depend on several factors like the molecular weight of

the biopolymer, the chemical composition of the backbone and side chains and their structure (Banerjee & Bhattacharya, 2012; Considine et al., 2011). Most of hydrocolloids can impart thickness to aqueous dispersions, whereas only some of them have the ability to form gels. This phenomenon involves the association or cross-linking of the polymer chains to form a three dimensional network that traps or immobilizes the water within it to form a rigid structure that is resistant to flow (Saha & Bhattacharya, 2010). Xanthan gum, guar gum, locust bean gum, and cellulose derivatives are some of the biopolymers generally used as thickening agents, whereas pectin, carrageenan, alginate, and gellan gum are employed to obtain gelled matrices.

Gellan gum is one of the most recent additions to the range of gelling agents available commercially. It has numerous existing and potential practical applications in food, cosmetics, toiletries, pharmaceuticals and microbiology (Lorenzo, Zaritzky, & Califano, 2013; Morris, Nishinari, & Rinaudo, 2012; Morrison, Clark, Chen, Talashek, & Sworn, 1999; Osmatek, Froelich, & Tasarek, 2014). Gellan gum is a linear and anionic heteropolysaccharide produced by the bacterium *Sphingomonas elodea*. It has a tetrasaccharide repeating sequence of: $\rightarrow 3$ - β -D-glucose-(1 \rightarrow 4)- β -D-glucuronic acid-(1 \rightarrow 4)- β -D-glucose-(1 \rightarrow 4)- α -L-rhamnose-(1 \rightarrow). The native polymer is high acyl gellan (HAG) containing O-5-acetyl and O-2-glyceryl groups on the (1 \rightarrow 3)-linked glucose residue. When exposed to alkali at high temperatures, both acyl groups are

* Corresponding author at: Universidad Nacional de La Plata—CONICET, Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), 47 y 116, 1900 La Plata, Buenos Aires, Argentina. Tel.: +54 221 4254853; fax: +54 221 4254853.

E-mail address: lorenzogabriel@gmail.com (G. Lorenzo).

hydrolyzed and the deacylated form of low acyl gellan (LAG) is obtained.

Either gellan type produces a thermo reversible gel when aqueous dispersions of it are heated and cooled. However, gel characteristic vary according to the O-acyl content of the polysaccharide. Elastic, soft gels are produced with HAG, while hard and firm gels are obtained with the low acyl specie. Through a blending of the two forms, a diverse range of textures can be achieved that encompass many of the textures produced by other hydrocolloids (Sworn, 2000)

Locust bean gum (LBG) is a water-soluble polysaccharide obtained from the seed endosperm of carob tree widely used in the food, pharmaceutical and cosmetic industries. LBG is a galactomannan containing (1 → 4)- β -D-mannopyranosyl backbone with attachment of (1 → 6)- α -D-galactose single units (Barak & Mudgil, 2014). LBG dissolves in hot water and forms a viscous solution with rheology typical of that of macromolecular solutions (Chen, Liao, Boger, & Dunstan, 2001; Doublier & Launay, 1981). Locust bean gum has been previously employed to produce multi-component gels in combination with carrageenan or konjac glucomannan (Brenner, Wang, Achayuthakan, Nakajima, & Nishinari, 2013; Dunstan et al., 2001). It was found that rheological and textural attributes were enhanced and a reduction of the syneresis of the gels was observed when LBG content was increased (Arda, Kara, & Pekcan, 2009; Chen et al., 2001). However, no studies were found of mixed gels containing gellan and locust bean gums.

It is well recognized that rheological measurements are appropriate tools for obtaining information about the organization of macromolecules in the medium, thus the correlation of microstructure information with rheological data is useful to understand the macroscopic behavior in terms of the microstructure organization.

The present study is aimed to determine the effect of composition and storage time on the rheological and optical attributes of multi-component gels containing locust bean gum, low acyl and high acyl gellan gums.

2. Materials and methods

2.1. Materials

Food grade commercial gellan gums of different acyl content were kindly provided by CP Kelco (San Diego, CA). Two types of gellan gum were used in the present study with different percentage of acyl substitution: high acyl content, HAG (KELCOGEL® LT 100) and low acyl content, LAG (KELCOGEL®). Food grade locust bean gum (LBG) was purchased from Sigma Chemical Co. (St. Louis, MO) and used without any pre-treatment. Analytical grade sodium acetate and acetic acid were used to buffer the pH of the hydrocolloids dispersions (Anedra, Argentina). Distilled and deionized water was used in all solutions.

2.2. Preparation of hydrocolloids dispersions

Aqueous acetate buffer (pH 4.5, 50 mM Na⁺) was used as solvent, considering that its dissociation constant is in close proximity with the pH selected.

Gellan and locust bean gums dispersions were prepared by dissolving the powder in the aqueous buffer at a controlled temperature of 25 °C and stirred overnight to ensure a complete hydration of the hydrocolloids. All samples prepared also contained sodium azide (0.01 g/100 g) as an antimicrobial agent. Three stock solutions were prepared, one for each hydrocolloid, at a concentration of 0.5 g/100 g. Using these solutions the studied mixtures were prepared.

2.3. Preparation of the gelled matrices

A previously employed protocol was followed to prepare all the measured samples (Lorenzo et al., 2013): hydrocolloids dispersions were mixed in the proper amount and the final gel was prepared by heating the dispersions up to 90 °C at a constant rate (2 °C min⁻¹), maintaining constant stirring. Solutions were weighed and acetate buffer was added to make up for any weight lost in evaporation. Analytical grade Calcium Chloride (5 mM), previously dissolved in the compensation solvent was added to the hot hydrocolloids solutions. After 5 min at 90 °C samples were rapidly cooled down to 20 °C (20 °C min⁻¹). To prevent water evaporation during storage, the gelled samples were kept within the molds and covered with plastic film; finally they were packed in tightly sealed containers and stored at 20 °C until they were measured.

2.4. Experimental design

A three-component simplex lattice mixture design was used to evaluate the effect of high acyl gellan (HAG: X₁), low acyl gellan (LAG: X₂), locust bean gum (LBG: X₃). Concentration of each hydrocolloid was modified in the range 0–0.5 (g/100 g). Component proportions were expressed as fractions of the mixture with a sum (X₁ + X₂ + X₃) of one. Levels of the factors and experimental design of the 10 combinations in terms of coded and uncoded variables are presented in Table 1. Centroid point formulation was prepared three times and all replicates were analyzed. To evaluate the effect of storage time on the rheological and optical properties, all of the samples were studied at 1, 5, 10, 20, 30, and 45 days. Two additional formulations were included to complete the rheological analysis (M11 and M12 in Table 1).

2.5. Rheological experiments

All rheological measurements were performed using a controlled stress rheometer Haake RS 600 (Thermoelectron, Karlsruhe, Germany). After positioning the sample on the sensor system, it was allowed to rest for 10 min before starting the corresponding measurement. In all cases samples were covered with a thin film of silicone oil to avoid evaporation during the measurements. The data was processed using the IRIS Rheo-hub 2007 program (Winter & Mours, 2006) to perform the spectra calculations.

Specimens to be analyzed were prepared by pouring 1.2 ml of fluid sample into individual cylindrical molds (35 mm diameter), followed by rapid cooling down to 20 °C. This procedure allowed us to obtain gelled samples of 1 mm thick (± 0.1 mm).

Small amplitude oscillatory shear analysis was conducted in triplicate for each formulation. Samples were loaded between plates and a temperature of 25 °C was fixed to run the assays. The linear viscoelastic region (LVR) was determined through stress sweep tests in the range 0.01–800 Pa, at a fixed frequency (6.28 rad s⁻¹ Hz). Afterwards, frequency sweeps (0.01–100 rad s⁻¹) were conducted within the LVR. Each sample was measured at least 12 h after its preparation.

2.6. Optical characterization

Global stability of the hydrocolloids matrices was determined by light scattering measurements using a vertical scan analyzer (QuickScan, Beckman Coulter; Fullerton, USA) during 45 days at room temperature. The sample to be analyzed was placed in a cylindrical glass measurement cell. Near the cell is a reading head composed of a pulsed near infrared light source ($\lambda = 850$ nm) and two synchronous detectors. The transmission (TR) detector receives the light, which goes through the sample (0°), while the back-scattering detector receives the light backscattered by the

sample (135°). The QuickScan head scans the entire height of the sample (about 60 mm). Measurements were performed at least in duplicate.

From the obtained profiles, mean values of the transmission in the 20–50 mm zone (TR_m), corresponding to the medium part of the tube, were obtained. From the TR_m values, the relative change in transmission was calculated as:

$$\Delta TR(t) = \frac{(TR_m(t) - TR_m(0))}{TR_m(0)} \quad (1)$$

where $TR_m(0)$ is the mean transmission at the initial time and $TR_m(t)$ is the value at different storage times.

2.7. Response surface methodology and statistical analysis

All statistical analysis, mixture design, generation of response surfaces, 3D and contour plots were accomplished using the Expert Design (trial version 7.1.6, Stat-Ease Inc., Minneapolis, USA) statistical software. Response surfaces were obtained using Scheffe's canonical special cubic equation for three components (Cornell, 2011):

$$Y = \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \sum_{j=i+1}^3 \beta_{ij} X_i X_j + \beta_{123} \prod_{i=1}^3 X_i \quad (2)$$

where Y is the response variable, X_i are the proportion of components and β_i , β_{ij} , and β_{123} were the linear, cross product and triple interaction coefficients, respectively. A backward stepwise multiple regression analysis was followed to determine the significant terms in Eq. (2). Differences in the computed parameters were considered significant when the computed probabilities were less than 0.05 ($P < 0.05$).

After model fitting was performed, residual analysis was conducted to validate the assumptions used in the analysis of variance. This analysis included calculating case statistics to identify outliers and examining diagnostic plots such as normal and residual plots. The proportion of variance explained by the polynomial models obtained was given by the multiple coefficient of determination, R^2 , and the adequacy of the model was verified using a "lack of fit" test.

3. Results and discussion

3.1. Rheological properties: Small amplitude oscillatory shear

Fig. 1 shows the frequency sweeps for the gelled matrices obtained from binary mixtures. It is important to point out that the formulation containing only locust bean gum (M3 in Table 1)

was not considered in the rheological analysis, since it behaved as diluted gum dispersion, very different from the viscoelastic characteristics of the rest of the studied systems. To compensate this missing point, two additional formulations were included in the vicinity of M3 to complete the rheological analysis (M11 and M12 in Table 1).

All formulations exhibited a mechanical spectrum characteristic of highly interconnected gel-like network structures, showing mainly an elastic behavior with G' scarcely dependent on the frequency and significantly higher than G'' . However, the shape of the spectrum was defined by the type of gellan gum used. Several authors have previously reported the high gelling ability of gellan gums even at very low concentration in the presence of divalent ions (Rodríguez-Hernández, Durand, Garnier, Tecante, & Doublier, 2003).

Mixtures of locust bean and high acyl gellan (Fig. 1b) showed a viscous modulus with a minimum value at intermediate frequencies. Above a certain probing frequency there is not enough time for the polymer chains to disentangle and the sample behaves rubber-like with G' nearly independent of ω (Graebing, Muller, & Paliere, 1993; Pötschke, Abdel-Goad, Alig, Dudkin, & Lellingner, 2004; Wang, Winter, & Auernhammer, 2014). This intermediate zone called "plateau region" is located between the "terminal" and the "transition" zones in the plot of G' and G'' vs. frequency (Ferry, 1980). This plateau region reflects the entropy elasticity of the temporary entangled polymer network. These gels showed a soft and flexible matrix and exhibited a decrease in their elastic behavior when the locust bean concentration increased. Conversely, gels containing LBG and low acyl gellan (Fig. 1c) exhibited a characteristic "true gel" behavior. G' and G'' curves were almost parallel independent of the oscillation frequency. The storage modulus was practically a decade higher than the loss modulus over the entire frequency range. This is a typical behavior of rigid systems with a hard and brittle structure previously described by several authors (Huang, Tang, Swanson, & Rasco, 2003; Kasapis et al., 1999; Lorenzo, Zaritzky, & Califano, 2011b; Rodríguez-Hernández et al., 2003).

Rheological results of mixtures with HAG and LAG gellan (Fig. 1a) show that formulations containing high content of HA gellan behaved as a gel with a tendency to flow at high frequencies, which could be interpreted as a matrix with tenuous and slightly interconnected networks. When the proportion of LAG increased, the structure of gellan gels seemed more homogeneous and the mechanical spectra showed a solid-like nature with practically independent values of G' with frequency.

The relaxation spectrum is vital not only for constitutive models but also for the insight into the properties of a viscoelastic material. Particularly, the generalized Maxwell model was used to fit the experimental data in the present work. In this model, the values of

Table 1
Composition of the studied samples using a simplex lattice mixture design.

Formulation	Coded variables			Hydrocolloids fraction (g/100 g)		
	X_1 : HAG	X_2 : LAG	X_3 : LBG	HAG	LAG	LBG
M1	1	0	0	0.5	0	0
M2	0	1	0	0	0.5	0
M3	0	0	1	0	0	0.5
M4	0.667	0.333	0	0.333	0.167	0
M5	0.667	0	0.333	0.333	0	0.167
M6	0	0.667	0.333	0	0.333	0.167
M7	0.333	0.667	0	0.167	0.333	0
M8	0.333	0	0.667	0.167	0	0.333
M9	0	0.333	0.667	0	0.167	0.333
M10	0.333	0.333	0.333	0.167	0.167	0.167
M11*	0.167	0	0.833	0.083	0	0.417
M12*	0	0.167	0.833	0	0.083	0.417

HAG: high acyl gellan; LAG: low acyl gellan; LBG: locust bean gum.

* Added formulations to complete rheological analyses of the gelled matrices.

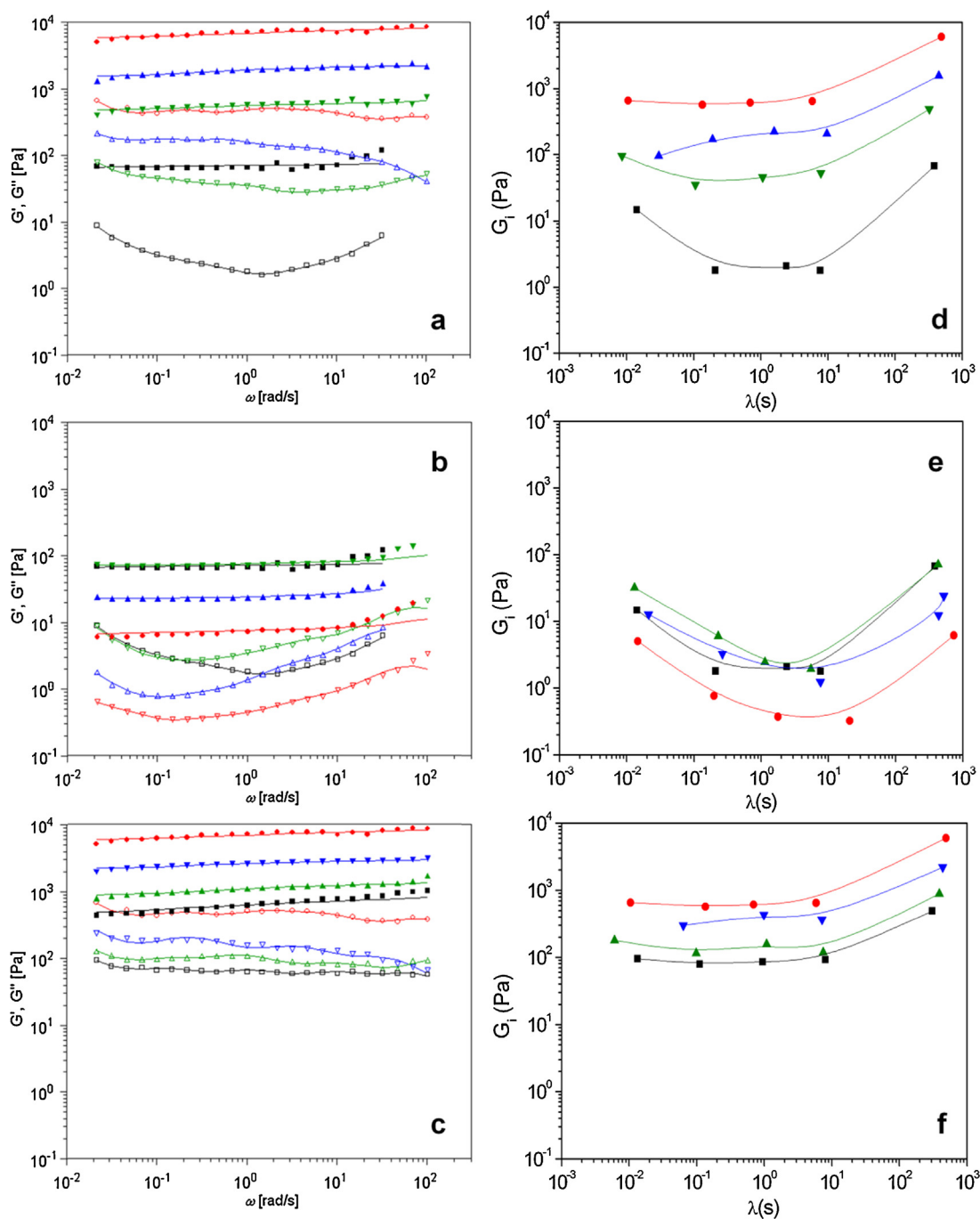


Fig. 1. (a–c) Mechanical spectra for binary mixtures, (d–f) relaxation spectra, Maxwell elements (G_i vs. λ_i). (a and d) mixtures of high (HAG) and low (LAG) acyl gellan gum, HAG content: 1 (■—M1), 0.66 (▼—M4), 0.33 (▲—M7), 0 (●—M2). (b and e) mixtures of HAG and locust bean gum (LBG), HAG content: 1 (■—M1), 0.66 (▼—M5), 0.33 (▲—M8), 0.166 (●—M11). (c and f) LAG-LBG mixtures, LBG content: 0.833 (■—M12), 0.67 (▼—M9), 0.33 (▲—M6), 0 (●—M2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the overall G' and G'' at any frequency are given by the sum of N contributions from N Maxwell elements in parallel. Each element is defined by the elastic response of the spring (G_i) and the relaxation time which is the ratio between the viscosity of the dashpot and the rigidity of the spring ($\lambda_i = \eta_i/G_i$). The following equations were fitted to the experimental data of the storage and loss moduli:

$$G'(\omega) = G_e + \sum_{i=1}^N G_i \frac{(\omega\lambda_i)^2}{1 + (\omega\lambda_i)^2} \quad (3)$$

$$G''(\omega) = \sum_{i=1}^N G_i \frac{(\omega\lambda_i)}{1 + (\omega\lambda_i)^2} \quad (4)$$

An iterative process using simultaneously Eqs. (3) and (4) was employed to minimize the sum of the square differences. The computed G_i and λ_i values were used to predict the storage and loss moduli.

The relaxation spectrum should pass through a minimum in the region where the storage modulus or relaxation modulus is flat, separating the two sets of relaxation times that correspond to

Table 2

Maximum storage time before the gels breakdown; critical strain amplitude at the initial time, and parameters of the kinetic analysis (Eq. (9)) for all the tested formulations. Standard deviations are shown between parentheses. Formulations are coded according to Table 1.

Formulation	Max. storage time (days)	Initial γ_{cr}	Parameters of destabilization kinetic		
			C_1	C_2	k (min ⁻¹)
M1	>45	0.120 (0.011)	NF	NF	NF
M2	>45	0.011 (0.0021)	NF	NF	NF
M4	>45	0.049 (0.0037)	-0.142 (0.011)	0.130 (0.016)	1.31×10^{-4} (1.91×10^{-5})
M5	30	0.225 (0.014)	-0.296 (0.039)	0.275 (0.039)	1.73×10^{-4} (1.70×10^{-5})
M6	30	0.019 (0.0022)	0.047 (0.0051)	-0.058 (0.0051)	5.10×10^{-5} (1.38×10^{-5})
M7	>45	0.021 (0.0013)	-0.090 (0.0028)	0.080 (0.0040)	7.66×10^{-5} (3.47×10^{-6})
M8	5	0.457 (0.032)	-0.224 (0.011)	0.195 (0.019)	3.15×10^{-4} (1.69×10^{-5})
M9	20	0.024 (0.0017)	0.134 (0.012)	-0.132 (0.011)	8.28×10^{-5} (3.21×10^{-6})
M10	>45	0.058 (0.0026)	-0.038 (0.0023)	0.055 (0.0026)	1.14×10^{-4} (2.40×10^{-5})
M11	1	0.847 (0.061)	-	-	-
M12	10	0.074 (0.0050)	-	-	-

NF: The model did not fit to the experimental data.

motions within entanglement strands and motions across entanglement loci (Ferry, 1980). In practice, the minimum is often blurred, probably in part because of distributions of entanglement spacings and some inhomogeneity of molecular weight even in relatively sharp polymer fractions.

The shape of the relaxation spectrum depended on the type of gellan gum in the formulation. Samples with LA gellan (Fig. 1f) showed a spectrum with higher values of G_i and a minimum region which extends to values of relaxation times below 10^{-2} s. Conversely, HA gellan samples (Fig. 1e) exhibited a clear minimum around 1 s. In HA-LA mixtures (Fig. 1d) this tendency was clearly observed. The minimum in G_i values shifted to shorter relaxation times and the region was widened as the concentration of low acyl gellan was increased.

In the HA-LBG mixtures, there was a slight shift of the minimum to higher λ_i values when LBG content increased. This shift toward higher relaxation times (or $\tan \delta$ shifting to lower frequencies) could be associated with an increase in the average molecular weight of the mixture as the concentration of locust bean gum increases (Ferry, 1980).

When hydrocolloid mixtures were analyzed during storage time it could be observed that almost every formulation exhibited a significant increment in G' and G'' during the first 10 days of storage and they remain practically within the same values during the whole storage time or until they broke down (destabilize). Visual inspection of the samples was used to determine which gels exhibited signs of syneresis or fracture; those samples were no longer measured (Table 2).

The increase of the storage moduli G' during storage of gellan gels has been attributed to the reinforcement of the structure by several authors. Sworn and Kasapis (1998) found that after more than 5 days of storage the structure of high sugar gellan mixtures was still consolidating, leading to a gradual increase in G' modulus. The transient nature of viscoelastic properties of diluted LAG gels was also studied by Pérez-Campos, Chavarría-Hernández, Tecante, Ramírez-Gilly, and Rodríguez-Hernández (2012), who reported that the transient behavior of G' depends on the calcium concentration in the matrix.

Storage of both low and high acyl gellan gels, as well as their mixtures, exhibited no sign of syneresis. This unique advantage of gellan gels compared to other polysaccharide gels was also found by Mao, Tang, and Swanson (2001). Conversely, samples containing binary mixtures of locust bean gum with either gellan type broke down before 45 days (Table 2). Moreover, gels obtained from HAG/LBG mixtures were the least stable samples, and their stability decreased when LBG content increased. In molecular terms, gels are metastable; three dimensional polymers networks formed by thermally-induced inter-chain association (Sanderson, 1990).

When two different biopolymers are thermodynamically incompatible, this segregative interaction can lead to resolution of the system into two layers or syneresis during storage. As it was found in the present work, inclusion of galactomannans, like guar or locust bean gums, was previously reported to form unstable gel matrices in combination with more than 2% of bovine gelatin (Harrington & Morris, 2009). Fig. 2 shows the values of G' and G'' at 1 Hz to analyze the effect of storage time in gelled matrices of HAG/LAG mixtures (formulations M1, M2, M4, and M7). Initially, mixtures of both gellan gums showed a slight synergism, but this effect was enhanced throughout the storage time, showing their large interaction after 10 days of storage. Particularly 2:1 mixtures of HAG:LAG showed this effect more markedly; but it was also observed in other formulations.

To characterize the behavior of the gelled matrices during storage, changes in rheological parameters could be analyzed using the theory of rubber elasticity (Flory, 1953; Lorenzo, Checmarev, Zaritzky, & Califano, 2011a; Schurz, 1991). It could be considered that the macromolecular network is composed of segments and junctions, and the junctions are the points where the intra- or intermolecular interactions are localized. The average conformation of a rubbery network under deformation is determined by both entropic and enthalpic contributions to the free energy change. The

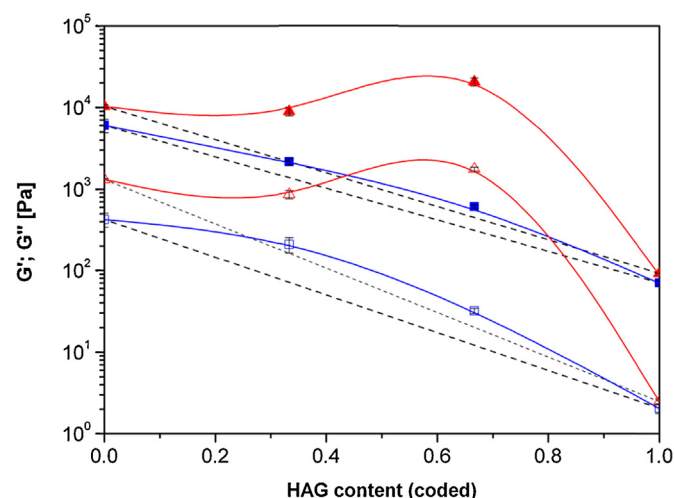


Fig. 2. Storage (G' : filled symbols) and loss (G'' : open symbols) moduli at 1 Hz as a function of high acyl gellan content in binary mixtures with low acyl gellan (expressed as coded variable according to Table 1). (■, □) initial time, (▲, △) 10 days of storage. Dashed straight lines correspond to a behavior with no interaction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

theory of rubber elasticity assumes that the enthalpic contribution may be neglected compared to the entropic one, and considers a network constituted of Gaussian chains (Kloek, Luyten, & Van Vliet, 1996; Kuang, Cheng, Zhao, & Li, 2006; Rao, 2007). Based on these concepts, the elastic modulus can be expressed as:

$$G_N^0 = \frac{cRT}{M_C} \left[1 - \frac{2M_C}{M} \right] \quad (5)$$

where G_N^0 is the plateau modulus (Pa), c is the concentration of the polymer in the network (in g m^{-3}), T the absolute temperature, R the ideal gas-law constant, M is the number average molecular weight for all hydrocolloids assuming a one component system, and M_C is the number-average molecular weight of the portion of the chain between two consecutive cross-links. The factor in brackets is the correction for free chain ends, but it could be neglected considering that $M \ll 2M_C$. Based on this relation and considering the equivalent network theory, it is possible to estimate the average distance between entanglement points, D_N (Schurz, 1991):

$$D_N = \sqrt[3]{\frac{6M_C}{\pi c N_L}} \quad (6)$$

where N_L is the Avogadro's Number and it was assumed an idealized network description made up by a collection of spheres whose diameter coincides with the average network mesh size D_N . The average network mesh size D_N (also known as correlation length) gives an indication of the mean 'pore-size' of the network, although the precise relation depends on the shape of the pores (Normand et al., 2003). Using the Maxwell elements previously determined, the plateau modulus was calculated as the sum of the elastic contribution (G_i) for each formulation at different storage times (Grassi et al., 2009; Pasut et al., 2008). From Eq. (6), the average polymeric network mesh (D_N) was determined and plotted in Fig. 3. It could be observed that D_N values at the initial time for all formulations spanned from 57.4 nm (M8) to 9.75 nm (M2). For carbodiimide cross-linked gelatin gels values of D_N around 2–5 nm were reported (Kuijpers et al., 1999). However, mesh size for 1.5% agarose gels was estimated over 120 nm by gel electrophoresis and wave length exponent method (Xiong et al., 2005), and 80 nm for 2% agarose gels (Normand et al., 2003). On the other hand, Shimizu, Brenner, Liao, and Matsukawa (2012) has estimated 30 nm the mesh size of 1% gellan gels by NMR, which is comparable to those values obtained in this work.

The studied formulations showed that increasing the content of low acyl gellan decreased the values of D_N . Noda et al. (2008) have found that the lower the acyl content of gellan gum the more continuous the network structures. They also reported that the lower the acyl content the more homogeneous and the more stretched the molecular bundles, resulting in an increase in the elasticity and the consistency of the gelled system.

Most of the tested systems showed a decrease in the average distance between two adjacent crosslinks with the storage time. This phenomenon may be associated with the continuous rearrangements in the matrix leading to a more ordered structure by growing the existent junction zones or the association of double helices into stable aggregates. It is worth mentioning that those formulations made with high acyl gellan and locust bean gum were the only ones that did not show this trend. Unlike the rest of the samples, these matrices showed systematic weakening during storage time until their complete breakdown. Additionally, these samples presented the highest D_N values, which have been previously related with the low connectivity of the gels (Coviello et al., 2013; Grassi et al., 2009; Kuijpers et al., 1999).

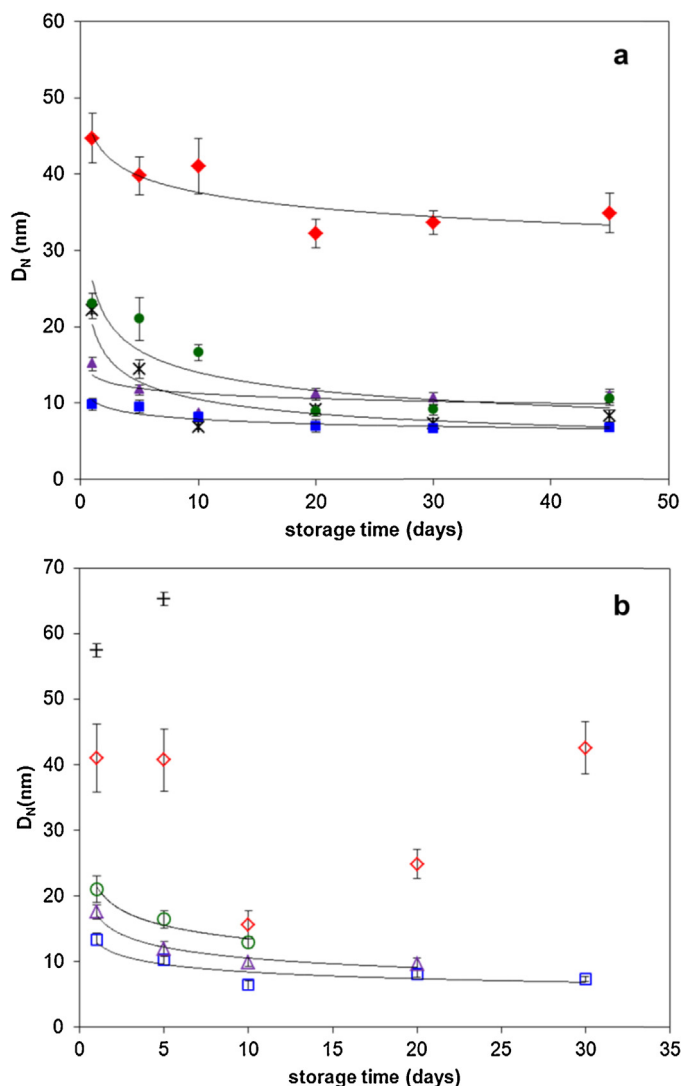


Fig. 3. Average distance between entanglement points (D_N) as a function of the storage time for all formulations. (a) M1- \blacklozenge -, M2- \blacksquare -, M4- \times -, M7- \blacktriangle -, M10- \bullet - (b) M5- \blacklozenge -, M6- \square -, M8- \times -, M9- \triangle -, M12- \circ -. Formulations are coded according to Table 1 and error bars correspond to SEM value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Rheological properties: Large amplitude oscillatory shear

Additionally, the strain dependence of the rheological parameters was evaluated inside and outside the linear viscoelastic range. As the strain amplitude increases, the rheological properties stay constant up to a critical strain amplitude (γ_{cr}), after which they deviate (Fig. 4). The region below the critical strain amplitude is defined as the linear region, and the region above the critical strain amplitude is called nonlinear. The dynamic test in the nonlinear region is often called as large amplitude oscillatory shear (LAOS) test, because the strain amplitude is not small anymore. The LAOS behavior has also been studied in depth but much less than the SAOS because nonlinear phenomena are more difficult to analyze (Hyun et al., 2011).

Hyun, Kim, Ahn, and Lee (2002) observed that the behavior of storage and loss moduli with strain variation could be classified by at least four types of strain-amplitude dependence for complex fluids, i.e., strain thinning (type I: G' and G'' decreasing); strain hardening (type II: G' and G'' increasing); weak strain overshoot (type III: G' decreasing, G'' increasing followed by decreasing); strong strain

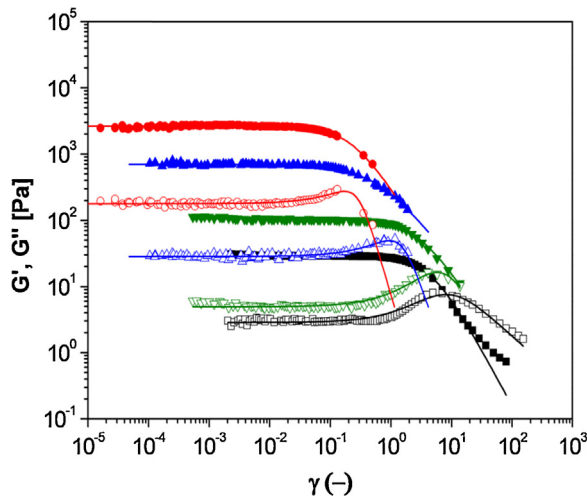


Fig. 4. Strain dependence of the storage (full symbols) and loss (open symbols) moduli for samples M4 (\blacktriangle), M5 (\blacktriangledown), M7 (\bullet), and M8 (\blacksquare), coded according to Table 1. Continuous lines correspond to the fitting using Eqs. (7) and (8). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

overshoot (type IV: G' and G'' increasing followed by decreasing). All gelled systems of this study could be classified as type III, since all of them presented a monotonic decay in the elastic modulus and a non-monotonic change in the loss modulus. For most of them, the loss modulus increased from its low strain value and then decreased with further increase in the strain amplitude. For the network model, type III is found when the creation rate of network junctions is smaller than their loss rate. Thus, the overshoot (i.e. local maximum of G'') may be regarded as arising from the balance between the formation and the destruction of the network junctions. Specifically, the maximum of G'' depends more on the creation rate than on the loss rate (Hyun et al., 2011). Fig. 4 shows as an example the strain dependence of both moduli for some of the studied mixtures. This response has been observed in a wide variety of soft materials with different characteristics and microstructures, such as emulsions (Bais, Trevisan, Lapasin, Partal, & Gallegos, 2005; Bower, Gallegos, Mackley, & Madiedo, 1999), gels and dispersions of different hydrocolloids (Kamble, Pandey, Rastogi, & Lele, 2013; Wyss et al., 2007), and starch pastes (Lawal et al., 2011; Utrilla-Coello et al., 2014), among others.

The strain dependence of G' and G'' can be fitted using a phenomenological equation. A damping function previously used by Soskey and Winter (1984) and then modified by Lapasin, Grassi, and Cocceani (2001) could be useful to describe and compare the behavior of gel matrices (Bais et al., 2005; De'Nobili, 2014; Oblonšek, Šostar-Turk, & Lapasin, 2003):

$$G' = G'_0 \frac{1 + a'\gamma}{1 + (b'\gamma)^{n'}} \quad (7)$$

$$G'' = G''_0 \frac{1 + a''\gamma}{1 + (b''\gamma)^{n''}} \quad (8)$$

where G'_0 and G''_0 represents the limiting values of the moduli (G' or G'') in the linear viscoelastic region and a' , a'' , b' , b'' , n' , and n'' are the fitting parameters. Considering that the storage modulus behaves like a monotonic decreasing function, Eq. (7) reduces to Soskey–Winter equation ($a' = 0$). Experimental data of each formulation at different storage time were satisfactorily fitted using Eqs. (7) and (8), as it can be seen in Fig. 4.

The critical strain (γ_{cr}) was arbitrarily associated with the condition that both moduli differ more than 5% from the corresponding linear values, and then it can be calculated from the equations

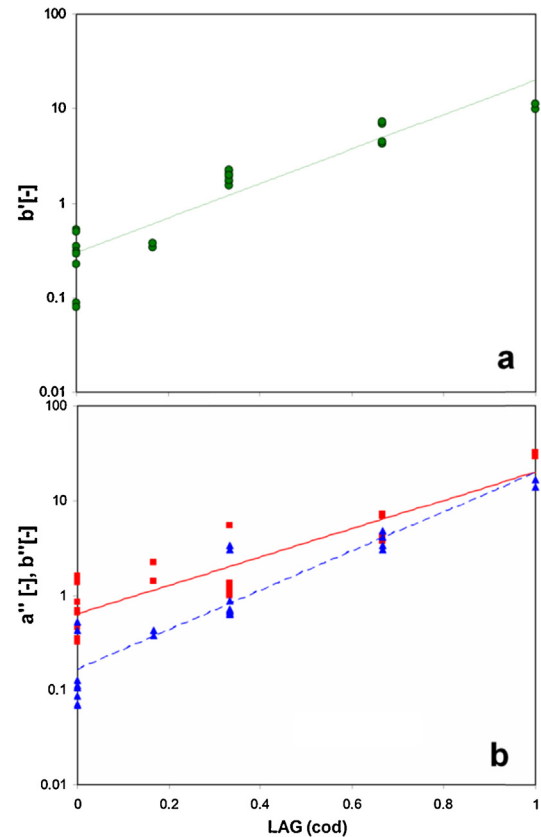


Fig. 5. Dependence of the parameters b' (a), a'' and b'' (b) of Eqs. (7) and (8) with low acyl gellan (LAG) concentration, expressed as a coded variable according to Table 1. Symbols: b' (\bullet), a'' (\blacksquare), b'' (\blacktriangle). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

parameters (Lapasin et al., 2001). Consequently, the critical strain values were determined using the average between the two values obtained from fitting G' and G'' (Table 2).

As it was expected, the limiting values of the moduli (G'_0 , G''_0) followed the same trend with composition and storage time as the mechanical spectra previously analyzed in Section 3.1 of the present paper. The other parameters of the model did not show significant changes with storage time. Nevertheless the hydrocolloids present in the matrix affected the strain dependence of the moduli. Fig. 5 reveals the overall behavior showing that b' , a'' , and b'' increased when LAG content was increased regardless the other hydrocolloids present in the matrix. This behavior leads to a marked decrease in the critical strain values with increasing LAG content, i.e. although gel matrices became more elastic with the presence of low acyl gellan it tended to break at smaller deformation values. Furthermore, the values of a'' were always higher than the ones of b'' , but the ratio (a''/b'') decreased when LAG fraction increased (Fig. 5b). This feature reflects that the overshoot in G'' values became less pronounced when low acyl gellan content was increased. Sim, Ahn, and Lee (2003) explained that the increase of G'' is partly related to the destruction of microstructures developed during the imposed oscillation, and the overshoot behavior is due to the reformation process of the clusters. In the present work, increasing LAG content produced a firmer gel matrix that could hinder the partial breakup of the structure and decrease the overshoot height leading to a strain thinning behavior due to layers sliding past each other in the flow direction. This behavior is in agreement with several authors who reported an increase in gel brittleness with increasing the low acyl gellan content (Bradbeer,

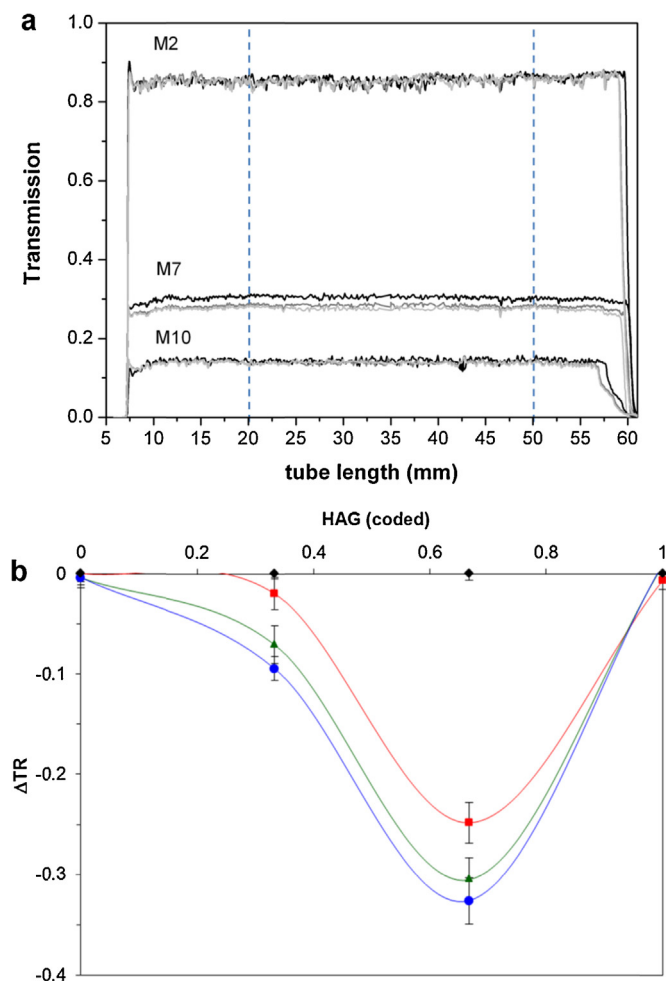


Fig. 6. (a) Transmission profiles of hydrocolloids mixtures at different storage times: initial time (—), 10 days (—), and 45 days (—). The region between dotted lines was selected to evaluate changes during storage. (b) Relative change in transmission (ΔTR) as a function of HAG content in binary mixtures with LAG (expressed as a coded variable according to Table 1). (■) initial time, (▲) 10 days (●), and 45 days of storage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Hancock, Spyropoulos, & Norton, 2014; Foo, Liong, & Easa, 2013; Kasapis et al., 1999; Sworn, 2000).

3.3. Optical characterization

The physical stability and optical changes in the gel matrices studied were characterized by multiple light scattering. This technique has been found to be useful to analyze the physical stability of several systems, mainly emulsions (García, Alfaro, Calero, & Muñoz, 2014; Márquez, Medrano, Panizzolo, & Wagner, 2010; Quintana, Lorenzo, Zaritzky, & Califano, 2007), but also for hydrocolloids mixtures (Closs, Conde-Petit, Roberts, Tolstoguzov, & Escher, 1999; Giancone, Torrieri, Masi, & Michon, 2009). This method was able to detect at an early stage the occurrence of different structural changes long before they could be observed by the naked-eye.

As a typical example, Fig. 6a shows the transmission profiles of three randomly selected formulations. The shape of the curves was qualitatively similar and the maximum transmission value was related to the hydrocolloids presents in the mixture. High acyl gellan formed cloudy gels with the lowest values of transmission ($TR_m = 0.032$), whereas the low acyl specimen produced practically transparent gels very rigid and brittle which displayed the highest transmission values ($TR_m = 0.846$).

To evaluate changes during storage, the relative change in the average transmission was calculated according to Eq. (1) for each formulation. Fig. 6b shows that changes in ΔTR for binary mixtures of HAG and LAG exhibited a similar behavior to the one detected in the mechanical spectra (Fig. 2). While no significant changes occurred in the transmission profile of the individual components during the 45 days of storage, a reduction of ΔTR was observed for the mixed matrices. These changes in the optical characteristics did not involve a macroscopic phase separation and they could be related to findings of gellan mixtures in previous research works (Kasapis et al., 1999; Matsukawa & Watanabe, 2007). Both studies used different techniques like DSC, circular dichroism, and NMR, and found that gelation of both hydrocolloids occurred independently; it means, that the double helix formation involves only the same kind of gellan chains even in the homogeneous solution of mixed gellan species. Rearrangements during storage of mixed gels could be related to the fact that high acyl and deacylated gellan do not form double helices incorporating strands of both types and this could lead to changes in the transmission profile as it was observed in the present work. This behavior was described by Morris (1990) as an “interpenetrating network structure”, where the two hydrocolloids remain intimately mixed and not confined to only part of the total volume, but form two separate gels permeating through one another (Brownsey & Morris, 1988; Morris et al., 2012).

3.4. Kinetic analysis

Further information on the influence of the hydrocolloid on the stability of the matrices was provided by a kinetic study. The relative change in the average transmission was plotted against the storage time and fitted to an exponential equation:

$$\Delta TR(t) = C_1 + C_2 \exp(-kt) \quad (9)$$

where C_1 , C_2 , and k are the parameters of the model.

All formulations showed a satisfactory fitting ($R^2 > 0.97$) to Eq. (9) except for M1 and M2 (i.e. systems containing only one type of gellan gum), which did not show variation in the transmission profile during storage and so a significant lack of fit was obtained with this model. Table 2 presents the parameters of Eq. (9) corresponding to the modeled samples. It is interesting to point out that for each formulation, coefficients C_1 and C_2 showed statistically the same value but with opposite sign, since according to the definition used in Eq. (1), at the initial time ΔTR has to be zero. Each type of gellan gum had a significant effect on a different parameter. Increasing high acyl gellan content significantly increased the value of C_2 , regardless the other hydrocolloid in the mixture. This corresponds to a larger difference between the initial and final value in the transmission profile, i.e. the greater the amount of HAG in the mixture the larger the extent of the optical changes in the matrix. On the other hand, the main effect on the kinetic coefficient k was due to the low acyl gellan content. Increasing the LAG concentration in the hydrocolloid mixture decreased the value of k (Table 2). This indicates that the use of LAG provided an improvement in the long-term stability of the matrices physical stability. A similar behavior was reported by García et al. (2014) when LAG and xanthan mixtures were used to stabilize o/w emulsion.

3.5. Stability of the ternary system

In order to fully understand the ternary mixed system, the response surface methodology was applied to the rheological and optical parameters (Fig. 7). Rheological data were transformed into a logarithmic scale to normalize the error distribution. Storage and loss moduli showed a qualitatively similar complex variation with composition (Fig. 7a and b). Both exhibited a strong dependence

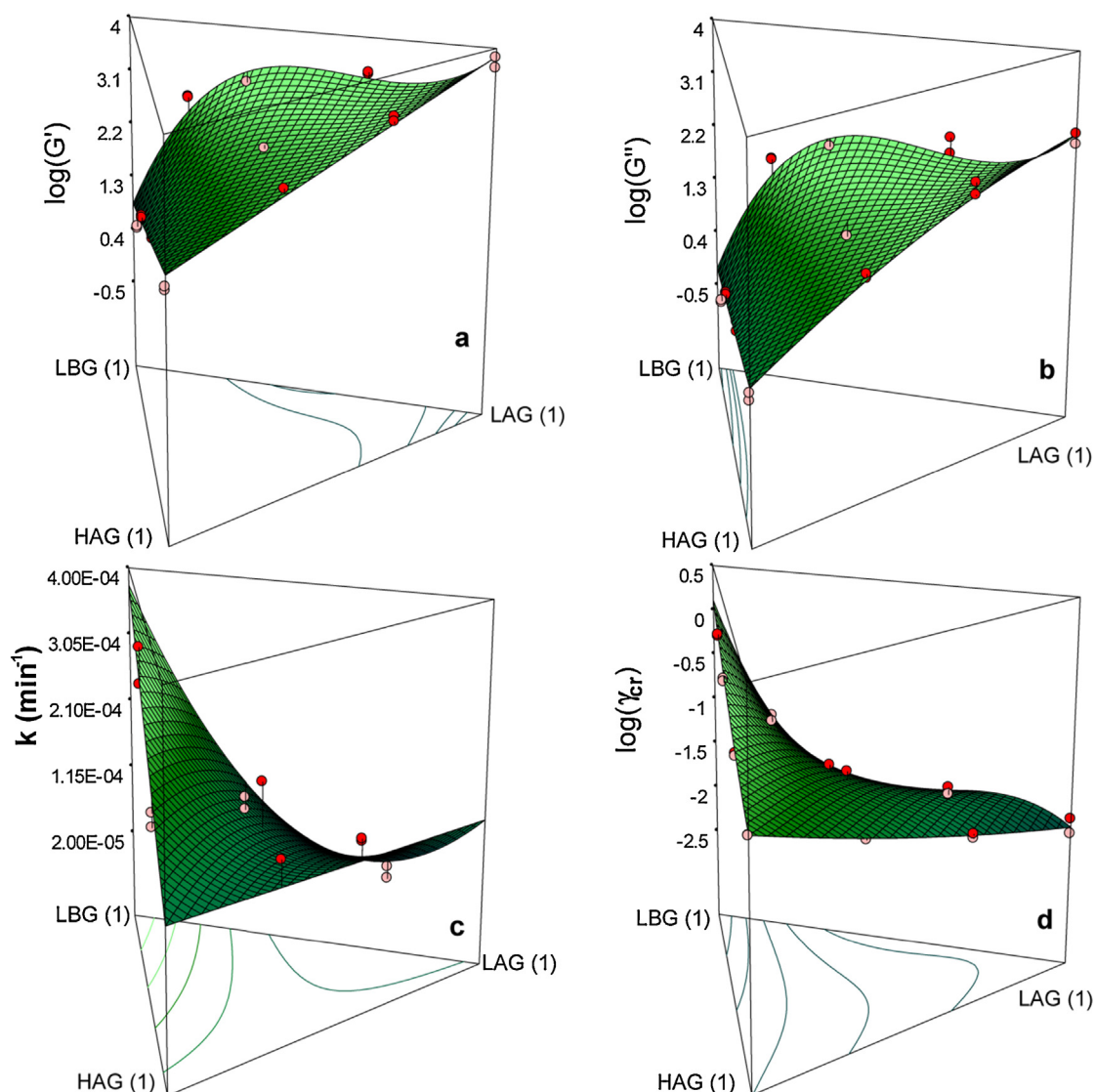


Fig. 7. Response surfaces for the initial values of (a) $\log(G'$ (at 1 Hz)), (b) $\log(G''$ (at 1 Hz)), (c) kinetic coefficient, k , obtained from Eq. (9), (d) logarithm of critical deformation (γ_{cr}).

on the low acyl gellan content and a significant contribution of the interaction $\text{LAG} \times \text{LBG}$. Conversely, while the increment of LAG in the matrix resulted in a firmer structure, the critical deformation of the linear viscoelastic range followed the opposite behavior, reaching values of γ_{cr} as low as 10^{-2} when the gels were composed only by the low acyl species.

Regarding the stability of the mixtures, the response surface shows that increasing the LAG content decreases the value of the kinetic constant, being the interaction $\text{LAG} \times \text{LBG}$ also significant (Fig. 7c).

It is worth to mention that binary mixtures containing LBG were destabilized before 45 days of storage (Table 2), whereas when the locust bean gum was used in a mixture with the two types of gellan gum simultaneously, the gelled matrix remained stable throughout the whole studied period.

Since physical gels are essentially non-equilibrium systems, the study of long time kinetic behavior seems often valuable and essential for practical application. In the present work, stable multi-component gels with a wide range of rheological and optical properties were obtained. Thus, the possibility to select and manipulate the desired characteristics of gelling systems

could be possible by choosing the appropriate mixture of components.

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