

## Rheological analysis of emulsion-filled gels based on high acyl gellan gum

Gabriel Lorenzo<sup>a,b,\*</sup>, Noemí Zaritzky<sup>a,b</sup>, Alicia Califano<sup>a</sup>

<sup>a</sup> Centro de Investigación y Desarrollo en Criotecología de Alimentos (CIDCA), CONICET – La Plata, Facultad de Ciencias Exactas, UNLP, 47 y 116, La Plata 1900, Argentina

<sup>b</sup> Departamento de Ingeniería Química, Facultad de Ingeniería, UNLP, Argentina

### ARTICLE INFO

#### Article history:

Received 6 February 2012

Accepted 27 August 2012

#### Keywords:

Gel emulsions

Gellan gum

Rheology

Relaxation time spectrum

### ABSTRACT

Emulsion-filled gels are widely used in cosmetic, food, and pharmaceutical industry. As rheological properties of these systems are strongly dependent on the properties of the gelled polymer network, rheological characteristics of gels containing high and low acyl gellan gum were analyzed. Under the processing conditions low acyl emulsions were unstable, thus in the present work the influence of oil and hydrocolloid concentrations on the viscoelastic behavior of emulsion-filled gels containing high acyl gellan gum was studied. Increasing gellan concentration (from 0.1 g/100 g to 0.5 g/100 g) produced stronger gels, while oil fraction (10 g/100 g–30 g/100 g) slightly affected the elastic behavior of the emulsions reinforcing the structure and the elastic characteristics of the gellan matrix. Sauter diameter ( $d_{32}$ ) was measured for all emulsions and an average value of 12  $\mu\text{m}$  was obtained. Rheological data (oscillatory and creep–recovery tests) were successfully modeled to interpret the structural characteristics of the gelled emulsions. The broadened Baumgaertel–Schausberger–Winter spectrum was used to represent the linear viscoelastic behavior of the continuous phase and the emulsified system, showing that the rheological behavior of the systems was controlled by the highly structured continuous phase rather than the contribution of filler lipid droplet in the emulsion. Relaxation spectra were validated using creep–recovery experiments. Regardless of hydrocolloid concentration, creep compliance of the gel emulsions decreased compared with their respective gels, showing that the inclusion of oil droplets produced a reinforcement of the structure and the gel strength of the matrix.

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### 1. Introduction

Gellan is an extracellular anionic polysaccharide produced by the bacterium *Sphingomonas elodea* (ATCC31461) formerly known as *Auromonas elodea* or *Pseudomonas elodea* on aerobic fermentation (Sworn, 2000). It has a linear tetrasaccharide repeating sequence of: [ $\rightarrow 3$ ]- $\beta$ -D-glucose-(1  $\rightarrow$  4)- $\beta$ -D-glucuronic acid-(1  $\rightarrow$  4)- $\beta$ -D-glucose-(1  $\rightarrow$  4)- $\alpha$ -L-rhamnose-(1  $\rightarrow$ ) (Jansson, Lindberg, & Sandford, 1983). The native polysaccharide, as biosynthesized, has an L-glyceryl substituent on O2 of the 3-linked glucose and about half the repeat units have an acetyl group on O6 of the same residue (Kuo, Mort, & Dell, 1986). Its industrial usefulness is due to its various functional properties. This gelling agent has the ability to form a variety of gel textures depending on combinations of polymer concentration, degree of substitution, type of cation, ionic strength, and temperature (Goh, Hainsman, & Singh, 2006; Ikeda, Nitta, Temsiripong, Pongsawatmanit, & Nishinari, 2004). Gellan

gum has been a subject of interest due to its thermal stability and its ability to form transparent gels, even at low concentrations (Rodríguez-Hernández, Durand, Garnier, Tecante, & Doublier, 2003). In addition, traditional gelling agents such as agarose and carrageenan show reduced gelling capacity at low pH, whereas gellan gum can form strengthened gels (Moritaka, Nishinari, Taki, & Fukuba, 1995; Yamamoto & Cunha, 2007).

Gellan gum may be used for producing emulsion-filled gels, which are widely used in cosmetic, food, and pharmaceutical industry. If the continuous phase of an emulsion or foam is a semisolid, these systems can be described as 'filled gels' or 'composite solids'. The aggregation and cross-linking of protein and polysaccharides molecules into three-dimensional solid-like networks ('gels') is one of the most important mechanisms for developing microstructure with desirable textural attributes. Due to the elastic characteristics of the gellan phase, oil droplets can be kept in suspension avoiding creaming.

The rheological properties and the breakdown behavior of gels filled with emulsion droplets can be varied by changing the rheology of the gel matrix, oil content, droplet size, and the interactions between oil droplets and gel matrix (Aguilera & Kinsella, 1991; Kim, Gohtani, & Yamano, 1996; Kim, Renkema, & van Vliet, 2001). In the specific

\* Corresponding author. Centro de Investigación y Desarrollo en Criotecología de Alimentos (CIDCA), CONICET – La Plata, Facultad de Ciencias Exactas, UNLP, 47 y 116, La Plata 1900, Argentina. Tel./fax: +54 221 4254853.

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

literature filled-gels rheology has been studied on systems containing proteins (Chen & Dickinson, 1998; Chen, Dickinson, & Edwards, 1999; Dickinson, 2012; Dickinson & Hong, 1995; Kim et al., 2001; van Vliet, 1988; Xiong, Aguilera, & Kinsella, 1991) or polysaccharides (Chojnicka, Sala, de Kruif, & van de Velde, 2009; Kim et al., 1996; Piazza & Benedetti, 2010; Sala, van Aken, Cohen Stuart, & van de Velde, 2007; Weiss, Scherze, & Muschiolik, 2005) in the continuous phase.

Information about the rheology of emulsion-filled gels may be used as an analytical tool to provide fundamental insights about the structural organization and interactions of the components, as it allows calculating the relaxation time spectrum of the emulsions (McClements, 1999; Quintana, Califano, Zaritzky, Partal, & Franco, 2002). Knowledge of an expression for the relaxation time spectrum of polymeric and complex materials is advantageous because of two reasons: it provides analytical expressions for the material functions which are quantitative (as storage and loss moduli data would yield) and it reduces the description of the linear viscoelastic behavior of any complex material to the determination of only a few material-specific parameters. The resulting pattern is expected to be universally valid in the linear region (Baumgaertel & Winter, 1989).

Rheology of gellan gum dispersions and gels has been extensively studied as affected by acylation degree, concentration, ionic strength, temperature, and type of cation present, among other variables (Goh et al., 2006; Jampen, Britt, Yada, & Tung, 2001; Morris, Nishinari, & Rinaudo, 2012; Rodríguez-Hernández et al., 2003). However, no studies on emulsion-filled gels formulated with gellan gum have been found.

As rheological properties of emulsion-filled gel are strongly dependent on the underlying properties of the gelled polymer network (Kokini & van Aken, 2006), the objectives of the present work were: i) to study the rheological properties of gellan gum gels to be used as continuous phases in emulsion-filled gels; ii) to analyze the influence of oil and hydrocolloid concentrations on the viscoelastic behavior of emulsion-filled gels formulated with high acyl gellan gum; iii) to model the experimental data (oscillatory and creep–recovery tests) in order to interpret the structural characteristics of the gelled emulsions; iv) to determine and validate the relaxation time spectra for the studied emulsions as well as the corresponding continuous phases containing high acyl gellan gum.

## 2. Experimental

### 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 (KELCOGEL® LT 100) and low acyl content (KELCOGEL®). Food grade gellan gum, ~90% dry matter, was used without any pre-treatment. Commercial 100% sunflower oil (Molinos Río de La Plata SACIFI, Buenos Aires) was purchased from a local supermarket and used without any further treatment. Polyoxyethylene sorbitan monooleate (Tween® 80) emulsifier was purchased from Sigma Chemical Co. (St. Louis, MO). Analytical grade sodium acetate and acetic acid were used to buffer the pH of the continuous phases (Anedra, Argentina). Distilled and deionized water was used in all solutions and emulsions.

### 2.2. Preparation of gellan gum solutions

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

Gellan gum solutions were prepared by dissolving the powder in the aqueous buffer at a controlled temperature (25 °C) and

stirred overnight to ensure a complete hydration of the hydrocolloid. Three gellan concentrations were studied: 0.1 g/100 g, 0.3 g/100 g, and 0.5 g/100 g.

Gellan gum aqueous phases were prepared by heating the dispersions up to 90 °C at a constant rate (2 °C min<sup>-1</sup>), 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), was dissolved in the compensation solvent and added to the hot gellan solutions.

All samples prepared also contained sodium azide (0.01 g/100 g) as an antimicrobial agent.

### 2.3. Preparation of emulsion-filled gels

Appropriate quantities of sunflower oil were mixed with the gellan gum solutions to give three different concentrations of disperse phase in the final emulsion: 10 g/100 g, 20 g/100 g, and 30 g/100 g.

Lab-scale manufacture of emulsions was carried out using Ultra Turrax T-25 homogenizer (Ika, Steufen, Germany). The emulsifier (Tween® 80) was included in the aqueous phase before the homogenization, at a concentration of 1 g/100 g. Sunflower oil was added to the aqueous phase containing the hydrocolloids at room temperature. Emulsions were homogenized at 11,500 rpm for 4 min without thermal control during homogenization. Afterward, emulsified systems were heated up to 90 °C (2 °C min<sup>-1</sup>), maintaining a constant stirring, then 5 mM CaCl<sub>2</sub> were added followed by rapid cooling down to 20 °C (20 °C min<sup>-1</sup>).

### 2.4. Droplet size determination

Sample droplet size and distribution were measured using a microscope coupled to a DC 100 camera (Leica Microscopy Systems Ltd., Heerbrugg, Switzerland). Three objective lenses (10×, 40×, and 100×) calibrated with an objective micrometer and appropriate software (Global Lab Image/2 3.7, Data Translation Inc.) were used. Aliquots of fresh samples were observed after a 1:10 dilution with aqueous acetate buffer. Digitization was based on gray level and the diameter was estimated based on the equivalent circular diameter. Over 500 droplets were measured to estimate the average droplet size using 5 fields per test.

Sauter average diameter ( $d_{32}$ ) was calculated for each sample as follows:

$$d_{32} = \frac{\sum_{i=1}^N (n_i d_i^3)}{\sum_{i=1}^N (n_i d_i^2)} \quad (1)$$

where  $d_i$  is the droplet diameter,  $N$  is the total number of droplets and  $n_i$  is the number of droplets having a diameter  $d_i$ .

### 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. Data were processed using the IRIS Rheo-hub 2007 program to perform the spectra calculations (Winter & Mours, 2006).

Specimens to be analyzed by creep and oscillatory experiments 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 ( $\pm 0.1$  mm).

### 2.5.1. Temperature sweep experiments

To determine the gelation point of the continuous phases, temperature sweep experiments were carried out. Concentric cylinders geometry was used for these runs (DIN 53018, bob diameter: 41 mm). Temperatures were ramped downward between 90 °C and 15 °C at a constant rate (i.e. 0.5 °C min<sup>-1</sup>) and frequency (6.28 rad/s) within the linear viscoelastic range. For each formulation, three repeated measurements were done and mean values were reported.

### 2.5.2. Creep–recovery experiments

Compliance measurements were carried out in both aqueous phases and emulsions using a serrated parallel plates geometry (35 mm of diameter) in order to avoid wall slip phenomena (Lorenzo, Zaritzky, & Califano, 2008). During the measurements the temperature was maintained constant at 25 °C by thermostatically controlling the temperature of the plates. The samples were loaded, a 1 mm gap between plates was fixed and a resting time of 600 s was adopted to relax any normal stresses induced during sample loading. In order to determine the linear viscoelastic region, stress sweeps between 0.1 and 100 Pa were performed. Creep–recovery tests were carried out within the linear viscoelastic region. The stress was applied instantly and maintained for a period of 1200 s. After removing stress, compliance was also measured during 800 s. The recovery phase permits access to lower shear rates and therefore, makes it possible to study the rheological behavior of the system while it is in a practically non-perturbed state. These measurements, through the creep compliance parameter ( $J$ ) as a function of time, discriminate between the elastic and viscous responses (Koop, de Oliveira Praes, Reicher, de Oliveira Petkowicz, & Meira Silveira, 2009). The compliance parameter ( $J$ ) is the resulting strain divided by the applied stress. All measurements were carried out in triplicate and average values were reported.

### 2.5.3. Oscillatory rheological analyses

Small amplitude oscillatory shear analysis was conducted in triplicate for both the gellan gels (aqueous phases) and the emulsion-filled gels. 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 at a fixed frequency (6.28 rad/s – 1 Hz). Afterward, frequency sweeps (0.01–100 rad/s) were conducted within the LVR. Dependence of the storage ( $G'$ ) and loss ( $G''$ ) moduli with frequency was obtained in all cases.

Each sample was measured at least 12 h after its preparation. This protocol was adopted in order to ensure a complete equilibration of the emulsion.

## 2.6. Statistical analysis

Analysis of variance was performed by using ANOVA procedures of the SYSTAT software (SYSTAT Inc., Evenston, IL, USA). Bonferroni's test was chosen for simultaneous pairwise comparisons. Differences in means and  $F$ -tests were considered statistically significant when  $P < 0.05$ .

## 3. Results and discussion

### 3.1. Rheological analysis of high and low acyl gellan gum gels

Gellan gum solutions were subjected to the gelation process described in Section 2.2, and the mechanical spectrum of each

sample is shown in Fig. 1. Three concentrations of hydrocolloid (0.1, 0.3, and 0.5 g/100 g) were studied for both low acyl and high acyl gellan gums. As expected, increasing hydrocolloid concentration produced a significant increase in  $G'$  and  $G''$  for both types of gellan gum. However, depending on the degree of substitution of the hydrocolloid, the systems showed qualitatively different behaviors. Fig. 1a shows the gels with low acyl gellan gum; regardless the content of hydrocolloid, all the systems exhibited a “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 hard and brittle structure previously described by several authors (Huang, Tang, Swanson, & Rasco, 2003; Kasapis et al., 1999; Rodríguez-Hernández et al., 2003). In contrast, gels containing high acyl gellan gum showed lower values of both moduli and a minimum in the viscous modulus ( $G''$ ) around 10<sup>-1</sup>–10<sup>0</sup> rad/s (Fig. 1b).

Temperature sweeps were performed on gellan gum dispersions to determine the gel point. Fig. 2 shows the complex viscosity as a function of temperature for all the tested continuous phases. During the cooling of the aqueous dispersions it was observed that the complex viscosity ( $\eta^* = ((G'^2 + G''^2)^{1/2}/\omega)$ ) remained almost invariant to a certain temperature value ( $T_{gel}$ , sol–gel transition) from which there was an abrupt increase in all dispersions considered. Both  $T_{gel}$  and the gel strength increased with hydrocolloid content in solution (Fig. 2). The gelation mechanism of gellan gum was proposed by Grasdalen and Smidsrod (1987). As a hot solution cools gellan gum undergoes a disorder–order transition. This transition has been attributed to a coil–helix transition (Morris et al., 2012; Morris, Rees, & Robinson, 1980; Sworn, 2000). In the case of low acyl gellan gum, gel promoting cations such as Na<sup>+</sup> and Ca<sup>2+</sup> induce aggregation of the gellan double helices to form a three-dimensional network. The acyl substituents have

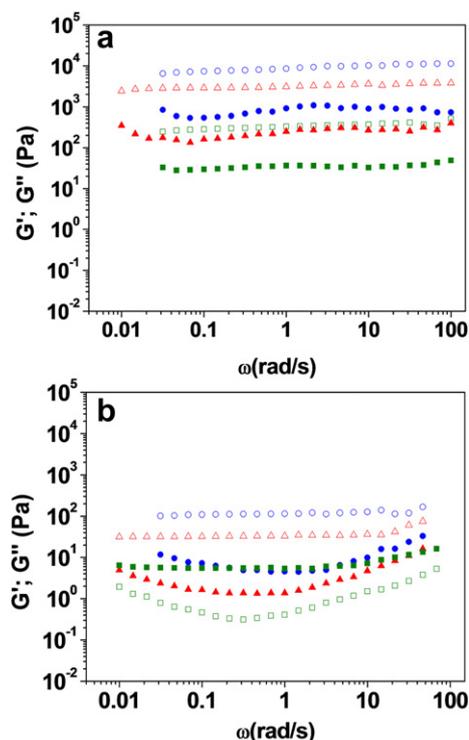
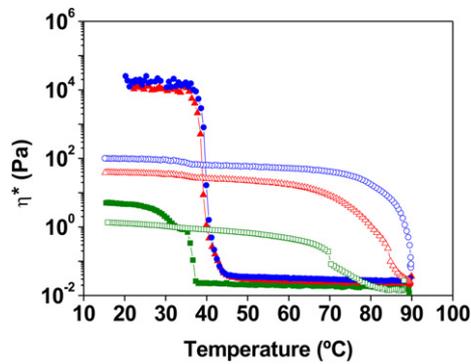


Fig. 1. Storage ( $G'$ ; open symbols) and loss ( $G''$ ; filled symbols) moduli as a function of frequency ( $\omega$ ) for gels obtained with low acyl (a) and high acyl (b) gellan gum and different hydrocolloid concentration. ( $\square$   $\blacksquare$ ) 0.1 g/100 g; ( $\triangle$   $\blacktriangle$ ) 0.3 g/100 g; ( $\circ$   $\bullet$ ) 0.5 g/100 g.



**Fig. 2.** Temperature dependence of the complex viscosity ( $\eta^*$ ) during cooling for solutions containing high (open symbols) and low (filled symbols) acyl gellan gum at different concentrations: (□ ■) 0.1 g/100 g; (△ ▲) 0.3 g/100 g; (○ ●) 0.5 g/100 g.

a marked effect on the structure of the gellan gum gels. The high acyl gellan gum undergoes a similar disorder to order transition as the solution is cooled, but further aggregation of the helices is limited by the presence of the acetyl group. The double helix structure of high acyl gellan is inherently more stable than that of the deacylated material, but it has little, if any, capacity for cation-mediated aggregation. The subsequent gels are, therefore, soft and elastic (Morris, Gothard, Hember, Manning, & Robinson, 1996).

### 3.2. Stability of emulsion-filled gels

It was observed that low acyl gellan gum emulsions creamed before reaching gelling temperature, while high acyl emulsions remained stable for several weeks. As an example, Fig. 3a shows o/w emulsions (20 g/100 g oil) using 0.3 g/100 g of high and low acyl gellan gum. The latter showed a marked creaming destabilization,

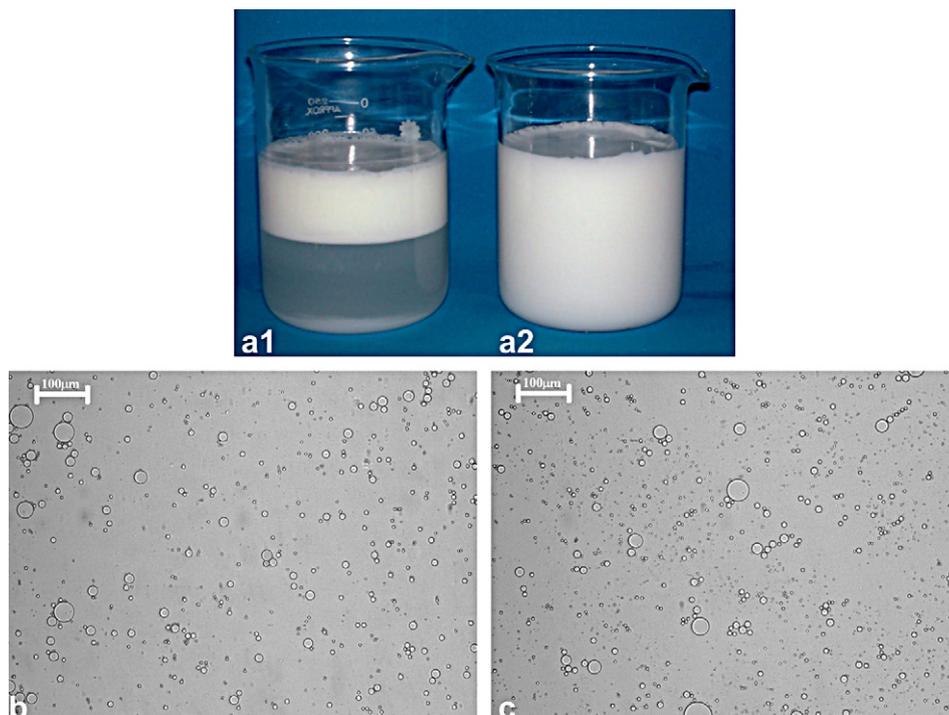
while the high acyl emulsion showed a uniform distribution of the dispersed phase throughout the volume. Gravitational separation depends on droplet size, oil fraction, and rheological properties of the continuous phase (McClements, 1999). In the present work droplet size distribution was not modified by oil fraction, type or content of gellan gum for all emulsions analyzed. Fig. 3b and c shows two different slightly flocculated emulsions. These flocs could be explained in terms of depletion flocculation caused by the presence of individual non-adsorbing molecules.

Average Sauter diameters ( $d_{32}$ ) ranged from 10.0 to 14.3  $\mu\text{m}$  (standard deviation = 2.4), however, not significant differences were observed between  $d_{32}$  values. Consequently, the differences in emulsion stability could not be explained in terms of the differences in droplet size; creaming stability depends on the capacity of the continuous phase to gel and immobilize oil droplets. Fig. 2 shows that gelation kinetic for both types of gellan gum is a fast process. However, it could be observed a marked difference in the sol–gel transition temperatures ( $T_{\text{gel}}$ ) of high and low acyl gellan gum solutions; the former showed  $T_{\text{gel}} > 70^\circ\text{C}$ , while the low acyl gellan gum solutions gelled below  $50^\circ\text{C}$ . As the high acyl gellan gum continuous phase gelled above  $70^\circ\text{C}$ , the droplets in the emulsion were rapidly entrapped by the high acyl gel matrix being unable to move upwards. On the other hand, in spite of the high cooling rate used, the low acyl continuous phase remained fluid for longer times allowing oil droplets to cream.

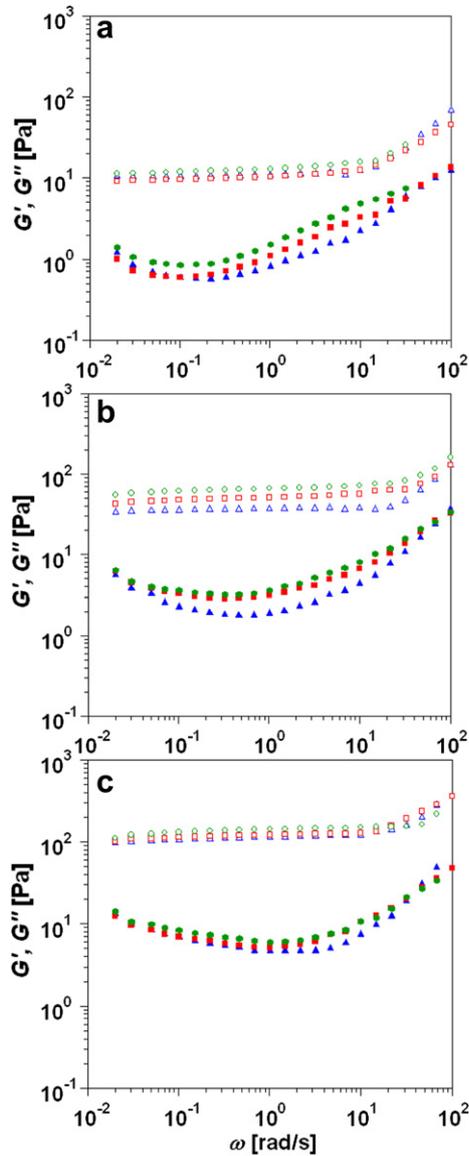
### 3.3. Dynamic rheology on emulsion-filled gels

The rheological analysis of emulsions was carried out only on those samples formulated with high acyl gellan gum which were stable under the present operating conditions.

The results of the dynamic measurements were expressed in terms of the storage modulus ( $G'$ ) and loss modulus ( $G''$ ) as a function of the angular frequency ( $\omega$ ). Fig. 4 shows the frequency



**Fig. 3.** Emulsion-filled gels formulated with an oil content of 20 g/100 g and 0.3 g/100 g: (a) Photographs of samples formulated with low acyl (a1) and high acyl (a2) gellan gum; (b, c) Micrographs of low and high acyl gellan gum o/w emulsions, respectively (dilution 1:10). Bars represent 100  $\mu\text{m}$ .



**Fig. 4.** Frequency sweeps for emulsion-filled gels containing 10 g/100 g ( $\Delta$ ,  $\blacktriangle$ ), 20 g/100 g ( $\square$ ,  $\blacksquare$ ), and 30 g/100 g ( $\circ$ ,  $\bullet$ ) lipid phase using (a) 0.1 g/100 g, (b) 0.3 g/100 g and (c) 0.5 g/100 g of high acyl gellan gum in the continuous phase.  $G'$ : open symbols;  $G''$ : filled symbols.

sweep curves for emulsion-filled gels obtained using different concentrations of high acyl gellan gum and sunflower oil. The curves were qualitatively similar for all the formulations assayed.  $G'$  was always higher than  $G''$  in the frequency range measured and the increase of the two moduli with frequency was small. As  $G' \gg G''$ , the material exhibited a solid behavior (i.e. deformation in the linear range will be essentially elastic or recoverable). The slight dependence of the storage modulus on the oscillation frequency is known as the “plateau region”. The plateau region is an intermediate zone of the mechanical spectra between the “terminal” and the “transition” zones (Ferry, 1980). It is characterized by a decrease in the slope of both moduli (lower than 1) and a possible minimum in the loss modulus ( $G''$ ).

The spectra show the characteristic behavior of viscoelastic solids, where the crosslinks between macromolecules are not permanent, but a dynamic equilibrium between formation and rupture of intermolecular interactions can exist contributing to the preservation of the structure during long observation times.

Although increasing dispersed phase concentration within the analyzed oil fraction (10 g/100 g–30 g/100 g) slightly increased storage and loss moduli, the most significant change was observed when gellan gum concentration increased (Fig. 4).

Several authors have been working on estimating the shear modulus of a composite gel. van der Poel (1958) proposed a particular formula to estimate the shear modulus of a composite gel, which was then simplified by Smith (1975). van Vliet (1988) extended this formula to predict the shear modulus of systems containing liquid emulsion droplets. Based on this theory, the modulus of filled gels ( $G'$ ) will depend on the relation between the stiffness of the gel matrix ( $G'_m$ ) and the stiffness of the filler particles ( $G'_f$ ) as follows (Chen & Dickinson, 1999; Smith, 1975):

$$\frac{G'}{G'_m} - 1 = \frac{15(1 - \nu_m)(M - 1)\phi}{Q - (8 - 10\nu_m)(M - 1)\phi} \quad (2)$$

where  $\phi$  is the oil fraction,  $\nu_m$  is the Poisson ratio of the matrix,  $M$  is the stiffness ratio ( $G'_f/G'_m$ ), and  $Q$  is defined by

$$Q = (8 - 10\nu_m)M + 7 - 5\nu_m \quad (3)$$

Following van Vliet (1988), the stiffness of the filler particles (oil droplets) can be estimated by the Laplace pressure, ( $G'_f = 2\gamma/R$ ), where  $\gamma$  is the interfacial tension and  $R$  is the droplet radius. For the gellan gel emulsions studied in this work, average droplet radius was 6.08  $\mu\text{m}$  and a value of 6 mN/m for the interfacial tension was used (Rosa, Sala, van Vliet, & van de Velde, 2006; Seta, Baldino, Gabriele, Lupi, & de Cindio, 2012), obtaining  $G'_f = 1.97$  KPa, always higher than the stiffness of the gel matrix ( $G'_m \leq 0.1$  KPa, as can be seen in Fig. 1b). Under these circumstances, it is expected that the filler particles deform less than the matrix and as a consequence, the modulus of the emulsion-filled gel is higher than  $G'_m$ . This behavior could be observed in Fig. 4 for gel emulsions containing high acyl gellan; when oil fraction increased a reinforcement of the filled gel was detected.

Using the calculated values  $Q$ ,  $M$ , and considering  $\nu_m = 0.5$ , Eq. (2) predicts a higher reinforcement of the emulsion-filled gels than the actually observed for all gellan gum concentrations. These differences could be explained considering that this theory assumes that the filler particles do not interact with each other, the matrix being completely adherent to the fillers (i.e., the fillers behave as bound fillers), and a homogeneous distribution of the filler through the gel network (Sala et al., 2007; van Vliet, 1988). In the present work gel emulsions were produced using a non-ionic water-soluble emulsifier (Tween 80), and this type of surfactant-coated emulsion droplets interacted only weakly with the gel matrix. Thus, increasing oil fraction strengthened the emulsion-filled gel but it was not as much as expected by the theory. Besides, the van der Poel theory was developed for homogeneous oil droplet distributions, since the emulsions embedded in the gellan gum matrix were widely polydisperse (Fig. 3b), this could be another factor that lead to differences between experimental and theoretical values of  $G'$ .

### 3.4. Creep and recovery analysis

Within the linear viscoelastic range a creep–recovery analysis was carried out on both the emulsion-filled gels and the corresponding high acyl gellan gels. Creep curves (compliance vs. time) were fitted to the Burgers model (Sherman, 1970; Steffe, 1996). To obtain a more accurate group of parameters for each sample, experimental data corresponding to both creep and recovery experiments were simultaneously fitted (Lorenzo, Checmarev, Zaritzky, & Califano, 2011; Steffe, 1996). Thus, according to Boltzmann superposition principle, the complete model is represented by the following equations:

$$J(t) = \begin{cases} J_0 + J_1 \left( 1 - \exp\left(\frac{-t}{(\lambda_{ret})_1}\right) \right) + \frac{t}{\eta_0} & t \leq t_1 \\ J_1 \exp\left(\frac{-t}{(\lambda_{ret})_1}\right) \left( \exp\left(\frac{-t_1}{(\lambda_{ret})_1}\right) - 1 \right) + \frac{t_1}{\eta_0} & t > t_1 \end{cases} \quad (4)$$

where  $J_0$  is the instantaneous compliance ( $\text{Pa}^{-1}$ ),  $\eta_0$  the viscosity of the Maxwell dashpot ( $\text{Pa s}$ ),  $J_1$  ( $\text{Pa}^{-1}$ ) and  $(\lambda_{ret})_1$  (s) are the compliance and the retardation time associated with the Kelvin–Voigt element, respectively, and  $t_1$  is the time at which the stress was removed.  $J_0$  represents the instantaneous elastic response of the system at  $t = 0$ ; the lower the value of  $J_0$ , the greater is the elasticity (Kaschta & Schwarzl, 1994).

Fig. 5 shows the creep and recovery curves for the different tested formulations. In all cases the Burgers model showed a satisfactory fitting to experimental data ( $r^2 > 0.89$ ).

While all formulations showed qualitatively the same behavior, there was a noticeable decrease in the compliance when hydrocolloid

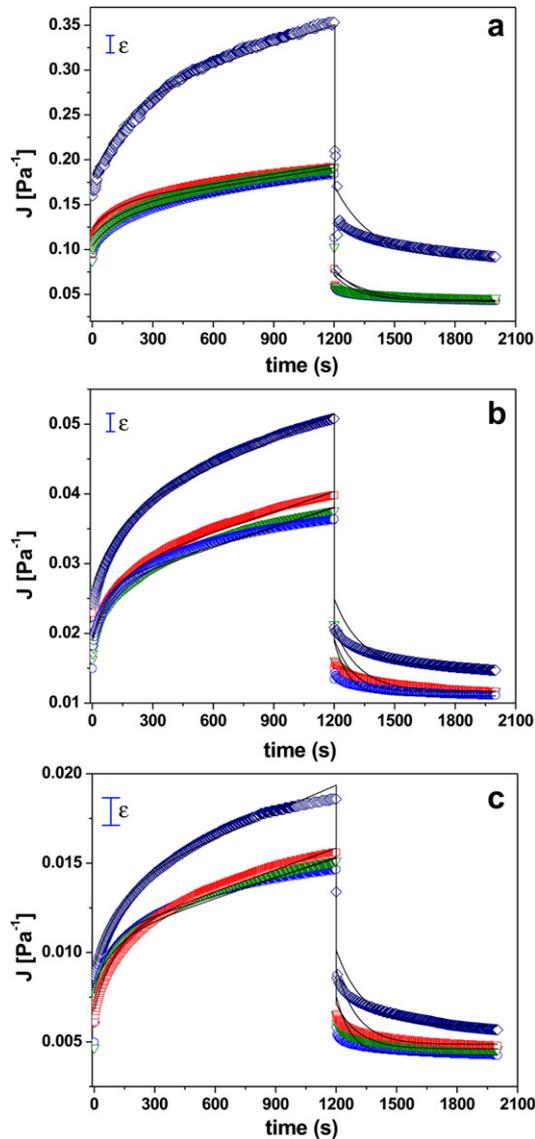


Fig. 5. Creep compliance curves for emulsion-filled gels containing 0 ( $\diamond$ ), 10 ( $\square$ ), 20 ( $\triangle$ ), and 30 g/100 g ( $\circ$ ) lipid phase using (a) 0.1 g/100 g, (b) 0.3 g/100 g and (c) 0.5 g/100 g of high acyl gellan gum in the continuous phase. Four parameters Burgers Model (continuous line). The vertical bar shows the average error of the compliance data ( $\epsilon$ ).

concentration increased. Particularly, increasing gellan gum content significantly decreased ( $P < 0.05$ ) the instantaneous compliance ( $J_0$ ). This parameter corresponds to the region where bonds between the different structural units of the emulsions are elastically stretched. When high acyl gellan gum concentration increased, the stiffness of the network increased resulting in less deformation at constant shear stress.

Regardless of hydrocolloid concentration, emulsified systems showed a considerable decrease in creep compliance compared with their respective gels. The presence of oil droplets led to a reinforcement of the matrix structure as has already been observed in the frequency sweep experiments (Fig. 4); it is important to notice that a further increase in the oil content (between 10 and 30 g/100 g) did not produce significant changes ( $P < 0.05$ ) in creep compliance curves.

Instantaneous and retarded compliances ( $J_0$  and  $J_1$ , respectively) of emulsion-filled gels showed a significant decrease when compared with their corresponding gels. Dolz, Hernández, and Delegido (2008) have reported similar results on gel emulsion using different hydrocolloids. Increasing oil droplets fraction led to an increased deformation difficulty of the viscoelastic system; consequently, a decrease in  $J_0$  and  $J_1$  values for the filled-gels is an expected behavior.

The retarded compliance  $J_1$  showed the same trend observed in  $J_0$ , but with values almost an order of magnitude lower (Table 1). This behavior is characteristic of highly flexible systems, where the material needs to deform initially before a flow is established and deforms again later (Jiménez-Avalos, Ramos-Ramírez, & Salazar-Montoya, 2005).

The retardation time associated with the Kelvin–Voigt element of the Burgers model,  $(\lambda_{ret})_1$ , showed a tendency to decrease both with increasing oil and gellan content. Conversely, the viscosity of the Maxwell dashpot ( $\eta_0$ ) showed a significant increase ( $P < 0.05$ ) with gellan concentration and non significant differences with oil concentration.

Although significant changes with the emulsion composition were observed in the Burgers model parameters, the percentage contribution of each element remained constant (Lorenzo et al., 2011). Instantaneous compliance ( $J_0$ ) represented the largest contribution, with an average 54% of the total deformation of the system, while  $J_1$  only explained 21% of the total compliance. All the emulsion-filled gels exhibited a recovery percentage above 70% of the total deformation. This is in agreement with results of dynamic tests where, although a marked increase in  $G'$  and  $G''$  was observed, the overall characteristics of the mechanical spectrum remained practically invariant.

Table 1

Predicted parameters of the four parameters Burgers model for gel like emulsions stabilized with different concentrations of high acyl gellan gum. Standard deviations are shown between parentheses.

Gellan gum [g/100 g]	Oil [g/100 g]	$J_0 (\times 10^3)$ [ $\text{Pa}^{-1}$ ]	$J_1 (\times 10^3)$ [ $\text{Pa}^{-1}$ ]	$(\lambda_{ret})_1$ [S]	$\eta_0 (\times 10^{-4})$ [ $\text{Pa s}$ ]
0.10	0	178 (2.4)	80.0 (2.1)	149 (44)	1.32 (0.04)
	10	121 (2.3)	30.6 (2.4)	119 (27)	2.79 (0.02)
	20	117 (1.2)	35.3 (3.4)	142 (26)	2.82 (0.06)
	30	115 (1.1)	32.5 (3.4)	118 (18)	2.82 (0.03)
0.30	0	27.0 (0.41)	9.30 (0.61)	130 (36)	7.91 (0.28)
	10	19.5 (0.17)	9.28 (1.0)	108 (18)	10.6 (0.36)
	20	19.1 (0.48)	8.32 (0.73)	99.4 (8.3)	10.6 (0.57)
	30	19.1 (0.34)	8.41 (0.75)	84.6 (11)	10.5 (0.38)
0.50	0	9.39 (0.14)	4.16 (0.11)	118 (33)	20.5 (0.28)
	10	7.17 (0.37)	3.88 (0.41)	110 (28)	24.7 (0.76)
	20	8.05 (0.21)	3.28 (0.69)	127 (36)	26.1 (0.72)
	30	7.84 (0.42)	3.46 (0.27)	80.9 (8.9)	28.5 (0.66)

### 3.5. Relaxation spectrum determination

The mathematical description of a viscoelastic material such as these gel emulsions is quite complex. The generation of such a model is not a straightforward operation since the constitutive equation required, universally valid for each fluid in every flow situation, has no standard form. Although some information can be gleaned from experiments, a complete description can only be obtained by solving the full set of governing equations.

During evaluation of mechanical properties, a broad spectrum of relaxation times ( $\lambda$ ) generating relaxation spectra ( $H(\lambda)$ ) has been observed for macromolecules (Winter, 1997). This relaxation spectrum is a fundamental quantity in the linear theory of viscoelastic materials and its shape is often correlated with specific molecular architectures (Honerkamp & Weese, 1993; Winter, 1997).

The dynamic moduli are related to the relaxation spectrum as follows:

$$G' = G_e + \int_{-\infty}^{+\infty} H(\lambda) \frac{(\omega\lambda)^2}{1 + (\omega\lambda)^2} d \ln \lambda \quad (5)$$

$$G'' = \int_{-\infty}^{+\infty} H(\lambda) \frac{(\omega\lambda)}{1 + (\omega\lambda)^2} d \ln \lambda \quad (6)$$

where,  $G_e$  represents the viscoelastic solid state of an additional spring attached in series to a Maxwell model that is called the equilibrium elasticity modulus (for viscoelastic liquids like the studied emulsions this parameter can be neglected).

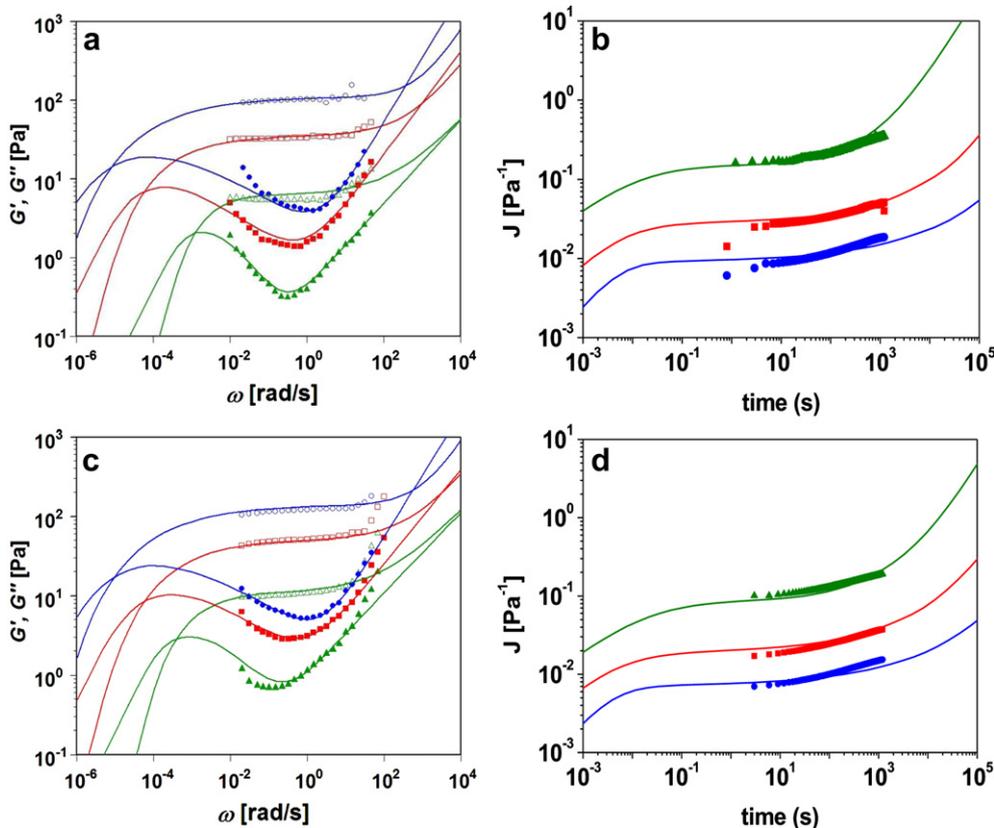
Using an appropriate representation of the relaxation time spectrum ( $H(\lambda)$ ), it is possible to model the dynamic moduli. Broadened Baumgaertel–Schausberger–Winter spectrum (denoted as “BSW spectrum”) has been successfully employed to represent the linear viscoelastic behavior of several polymeric and emulsified systems (Baumgaertel & Winter, 1992; Bengoechea, Puppo, Romero, Cordobés, & Guerrero, 2008; Franco, Berjano, & Gallegos, 1997; Lorenzo et al., 2011; Quintana et al., 2002).

$$H(\lambda) = G_N^0 \left[ H_g \left( \frac{\lambda}{\lambda_0} \right)^{-n_0} + n_e \left( \frac{\lambda}{\lambda_e} \right)^{n_e} \right] \exp \left( - \left( \frac{\lambda}{\lambda_{\max}} \right)^\beta \right) \quad (7)$$

where  $G_N^0$  is the plateau modulus,  $H_g$  is the glass-transition front factor,  $n_0$  and  $n_e$  are the slopes of the spectrum in the high frequency glass transition and entanglement regimes respectively,  $\lambda_0$  is the crossover time to the glass transition,  $\lambda_e$  the relaxation time corresponding to polymer chains with entanglement molar mass, and  $\lambda_{\max}$  is the longest relaxation time. The exponent  $\beta$  controls the sharpness of the cut-off of the spectrum.

The satisfactory agreement between experimental oscillatory data and predictions calculated using the BSW model is shown in Fig. 6 (a and c) for both high acyl gellan gels and emulsion-filled gels containing 20 g/100 g of sunflower oil.

It could be noted a significant increase of the plateau modulus with gellan concentration, while increasing dispersed phase content did not significantly affect  $G_N^0$  (Table 2). This could be explained considering that the rheological behavior of the systems is controlled by the highly structured continuous phase rather than the contribution of filler lipid droplet in the emulsion, as was previously pointed out. It was observed a relationship between  $G_N^0$



**Fig. 6.** Comparison between experimental data and the prediction from broadened BSW model for (a, c)  $G'$  (open symbols) and  $G''$  (filled symbols) and (b, d) Creep compliance. Data corresponded to: (a, b) continuous phases (high acyl gellan gels) and (c, d) emulsion-filled gels containing 20% oil with different hydrocolloids concentration: 0.1 g/100 g (triangles); 0.3 g/100 g (squares); 0.5 g/100 g (circles).

**Table 2**

Predicted parameters of the Baumgaertel–Schausberger–Winter model for gel emulsions (20% oil) and the aqueous phases stabilized with different gellan gum contents.

Gellan gum [g/100 g]	0.1		0.3		0.5	
	0.0	20	0.0	20	0.0	20
$G_N^0$ [Pa]	1.23	2.02	3.28	6.90	26.42	38.13
$\lambda_e$ [s]	18.12	42.0	23.66	55.2	203.7	200.0
$\lambda_0$ [s]	6.07	7.99	0.341	0.301	0.020	0.027
$\lambda_{max}$ [s]	72.5	94.7	$2.41 \times 104$	$9.23 \times 103$	$2.45 \times 104$	$1.00 \times 104$
$n_e$	0.695	0.730	0.357	0.41	0.311	0.330
$n_0$	0.546	0.53	0.639	0.603	0.840	0.800
$H_g$	0.047	0.074	0.198	0.246	0.191	0.138
$\beta$	0.221	0.330	0.830	0.550	0.370	0.320

and gellan content “ $c$ ” ( $G_N^0 \propto c^2$ ) similar to the tendency found in other polymers (Ferry, 1980; Graessley, 1974).

The evident displacement of  $\lambda_e$  to shorter relaxation times stands for an increasing molecular mobility (Table 2). The spacing between the crossover time to the glass transition ( $\lambda_0$ ) and the terminal relaxation time ( $\lambda_e$ ) is a measure of the width of the plateau zone on the logarithmic time or frequency scale. It showed a significant increment with the gellan concentration as was reported for other polymers (Ferry, 1980).

In an entirely analogous manner to the relaxation modulus, the continuous retardation spectrum ( $L(\lambda)$ ) could be defined in terms of creep behavior based on the Generalized Burgers model (Ferry, 1980; Steffe, 1996). Since there is an interrelation between the relaxation ( $H(\lambda)$ ) and retardation ( $L(\lambda)$ ) spectra, when  $H(\lambda)$  was obtained from the oscillatory data the retardation could be readily determined (Ferry, 1980). Once the retardation spectrum was known, the creep-compliance behavior was predicted. Fig. 6 (b and d) shows the predicted compliance for emulsion-filled gels as well as the experimental data.

Any dynamic data measured in this work have been successfully converted into the time domain by the application of BSW model. It is a useful tool, especially for establishing a rheological data bank and analyzing viscoelastic experiments.

#### 4. Conclusions

The present work analyzed the viscoelastic properties of gel emulsions formulated with high and low acyl gellan. Only high acyl gellan formed stable gel emulsions. Image analysis revealed that these gel emulsions were highly polydisperse and droplet size distribution was not affected by gum or lipid content.

The elastic properties of the emulsion-filled gels were mainly affected by gum content with stronger matrix reinforcement at higher gellan concentration. The influence of the oil content on the viscoelastic behavior of the filled gel matrix could be explained in terms of the stiffness ratio ( $G_f'/G_m'$ ) between oil droplets and the gellan matrix.

The experimental oscillatory data of the systems were satisfactorily modeled using the broadened BSW equation to predict the mechanical relaxation spectrum in the linear viscoelastic range. Creep compliance was predicted from this spectrum and compared with experimental data to confirm the correct fitting of the mechanical spectrum. Regardless of hydrocolloid concentration, creep compliance of the gel emulsions decreased compared with their respective gels, showing that the inclusion of oil droplets produced a reinforcement of the structure and the gel strength of the matrix.

#### Acknowledgments

The authors are grateful to CP Kelco (San Diego, CA), who kindly provided the gellan gum used for this study. The financial support of the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica, and Universidad Nacional de La Plata are also acknowledged.

#### References

- Aguilera, J. M., & Kinsella, J. E. (1991). Compression strength of dairy gels and microstructural interpretation. *Journal of Food Science*, 56(5), 1224–1228.
- Baumgaertel, M., & Winter, H. H. (1989). Determination of discrete relaxation and retardation time spectra from dynamic mechanical data. *Rheologica Acta*, 28, 511–519.
- Baumgaertel, M., & Winter, H. H. (1992). Interrelation between continuous and discrete relaxation time spectra. *Journal of Non-Newtonian Fluid Mechanics*, 44, 15–36.
- Bengochea, C., Puppo, M. C., Romero, A., Cordobés, F., & Guerrero, A. (2008). Linear and non-linear viscoelasticity of emulsions containing carob protein as emulsifier. *Journal of Food Engineering*, 87, 124–135.
- Chen, J., & Dickinson, E. (1998). Viscoelastic properties of heat-set whey protein emulsion gels. *Journal of Texture Studies*, 29, 285–304.
- Chen, J., & Dickinson, E. (1999). Effect of surface character of filler particles on rheology of heat-set whey protein emulsion gels. *Colloids and Surfaces B: Biointerfaces*, 12, 373–381.
- Chen, J., Dickinson, E., & Edwards, M. (1999). Rheology of acid induced sodium caseinate stabilized emulsion gels. *Journal of Texture Studies*, 30(4), 377–396.
- Chojnicka, A., Sala, G., de Kruijff, C. G., & van de Velde, F. (2009). The interactions between oil droplets and gel matrix affect the lubrication properties of sheared emulsion-filled gels. *Food Hydrocolloids*, 23, 1038–1046.
- Dickinson, E. (2012). Emulsion gels: the structuring of soft solids with protein-stabilized oil droplets. *Food Hydrocolloids*, 28, 224–241.
- Dickinson, E., & Hong, S. T. (1995). Influence of water-soluble non-ionic emulsifier on the rheology of heat-set protein stabilised emulsion gels. *Journal of Agricultural and Food Chemistry*, 43, 2560–2566.
- Dolz, M., Hernández, M. J., & Delegido, J. (2008). Creep and recovery experimental investigation of low oil content food emulsions. *Food Hydrocolloids*, 22, 421–427.
- Ferry, J. D. (1980). *Viscoelastic properties of polymers*. New York: John Wiley & Sons.
- Franco, J. M., Berjano, M., & Gallegos, C. (1997). Linear viscoelasticity of salad dressing emulsions. *Journal of Agriculture and Food Chemistry*, 45, 713–719.
- Goh, K. K. T., Hainsman, D. R., & Singh, H. (2006). Characterization of a high acyl gellan polysaccharide using light scattering and rheological techniques. *Food Hydrocolloids*, 20, 176–183.
- Graessley, W. W. (1974). The entanglement concept in polymer rheology. *Advances in Polymer Science*, 16, 1–179.
- Grasdalen, H., & Smidsrod, O. (1987). Gelation of gellan gum. *Carbohydrate Polymers*, 7, 371–393.
- Honerkamp, J., & Weese, J. (1993). A nonlinear regularization method for the calculation of relaxation spectra. *Rheologica Acta*, 32, 65–73.
- Huang, Y., Tang, J., Swanson, B. G., & Rasco, B. A. (2003). Effect of calcium concentration on textural properties of high and low acyl mixed gellan gels. *Carbohydrate Polymers*, 54(4), 517–522.
- Ikeda, S., Nitta, Y., Tamsiripong, T., Pongsawatmanit, R., & Nishinari, K. (2004). Atomic force microscopy studies on cation-induced network formation of gellan. *Food Hydrocolloids*, 18, 727–735.
- Jampen, S., Britt, I. J., Yada, S., & Tung, M. A. (2001). Rheological properties of gellan gels containing filler particles. *Journal of Food Science*, 66, 289–293.
- Jansson, P. E., Lindberg, B., & Sandford, P. A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*. *Carbohydrate Research*, 124, 135–139.
- Jiménez-Avalos, H. A., Ramos-Ramírez, E. G., & Salazar-Montoya, J. A. (2005). Viscoelastic characterization of gum arabic and maize starch mixture using the Maxwell model. *Carbohydrate Polymers*, 62, 11–18.
- Kasapis, S., Giannouli, P., Hember, M. W. N., Evageliou, V., Poulard, C., Bourgeois, B. T., et al. (1999). Structural aspects and phase behaviour in deacetylated and high acyl gellan systems. *Carbohydrate Polymers*, 38, 145–154.
- Kaschta, J., & Schwarzl, E. R. (1994). Calculation of discrete retardation spectra from creep data—II. Analysis of measured creep curves. *Rheologica Acta*, 33, 530–541.
- Kim, K. H., Gohtani, S., & Yamano, Y. (1996). Effects of oil droplets in physical and sensory properties of o/w emulsion agar gels. *Journal of Texture Studies*, 27, 655–670.
- Kim, K. H., Renkema, J. M. S., & van Vliet, T. (2001). Rheological properties of soybean protein isolate gels containing emulsion droplets. *Food Hydrocolloids*, 15, 295–302.
- Kokini, J., & van Aken, G. (2006). Discussion session on food emulsions and foams. *Food Hydrocolloids*, 20, 438–445.
- Koop, H. S., de Oliveira Praes, C. E., Reicher, F., de Oliveira Petkowicz, C. L., & Meira Silveira, J. L. (2009). Rheological behavior of gel of xanthan with seed

- galactomannan: effect of hydroalcoholic–ascorbic acid. *Materials Science and Engineering C*, 29, 559–563.
- Kuo, M. S., Mort, A. J., & Dell, A. (1986). Identification and location of L-glycerate, an unusual acyl substituent in gellan gum. *Carbohydrate Research*, 156, 173–187.
- Lorenzo, G., Checmarev, G., Zaritzky, N., & Califano, A. (2011). Linear viscoelastic assessment of cold gel-like emulsions stabilized with bovine gelatine. *LWT – Food Science and Technology*, 44, 457–464.
- Lorenzo, G., Zaritzky, N., & Califano, A. (2008). Modeling rheological properties of low-in-fat o/w emulsions stabilized with xanthan/guar mixtures. *Food Research International*, 41, 487–494.
- McClements, D. J. (1999). *Food emulsions: Principles, practice and techniques*. Boca Raton: CRC Press.
- Moritaka, H., Nishinari, K., Taki, M., & Fukuba, H. (1995). Effects of pH, potassium chloride, and sodium chloride on the thermal and rheological properties of gellan gum gels. *Journal of Agricultural and Food Chemistry*, 43, 1685–1689.
- Morris, E. R., Gothard, M. G. E., Hember, M. W. N., Manning, C. D., & Robinson, G. (1996). Conformational and rheological transitions of welan, rhamsan and acylated gellan. *Carbohydrate Polymers*, 30, 165–175.
- Morris, E. R., Nishinari, K., & Rinaudo, M. (2012). Gelation of gellan – a review. *Food Hydrocolloids*, 28, 373–411.
- Morris, E. R., Rees, D. A., & Robinson, G. (1980). Cation-specific aggregation of carrageenan helices: domain model of polymer gel structure. *Journal of Molecular Biology*, 138(2), 349–362.
- Piazza, L., & Benedetti, S. (2010). Investigation on the rheological properties of agar gels and their role on aroma release in agar/limonene solid emulsions. *Food Research International*, 43, 269–276.
- van der Poel, C. (1958). On the rheology of concentrated dispersion. *Rheologica Acta*, 1, 198–205.
- Quintana, M., Califano, A., Zaritzky, N., Partal, P., & Franco, J. M. (2002). Linear and nonlinear viscoelastic behavior of oil-in-water emulsions stabilized with polysaccharides. *Journal of Texture Studies*, 33(3), 215–236.
- Rodríguez-Hernández, A. I., Durand, S., Garnier, C., Tecante, A., & Doublier, J. L. (2003). Rheology-structure properties of gellan systems: evidence of network formation at low gellan concentrations. *Food Hydrocolloids*, 17, 621–628.
- Rosa, P., Sala, G., van Vliet, T., & van de Velde, F. (2006). Cold gelation of whey proteins emulsions. *Journal of Texture Studies*, 37, 516–537.
- Sala, G., van Aken, G. A., Cohen Stuart, M. A., & van de Velde, F. (2007). Effect of droplet-matrix interactions on large deformation properties of emulsion-filled gels. *Journal of Texture Studies*, 38(4), 511–535.
- Seta, L., Baldino, N., Gabriele, D., Lupi, F. R., & de Cindio, B. (2012). The effect of surfactant type on the rheology of ovalbumin layers at the air/water and oil/water interfaces. *Food Hydrocolloids*, 29, 247–257.
- Sherman, P. (1970). *Industrial rheology*. New York: Academic Press.
- Smith, J. C. (1975). Simplification of the van der Poel's formula for the shear modulus of particulate composite. *Journal of Research of the National Bureau of Standards*, 79A, 419–423.
- Steffe, J. F. (1996). *Rheological methods in food process engineering*. East Lansing: Freeman Press.
- Sworn, G. (2000). Gellan gum. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 117–135). Cambridge: Woodhead Publishing Limited.
- van Vliet, T. (1988). Rheological properties of filled gels. Influence of filler matrix interaction. *Colloid and Polymer Science*, 266, 518–524.
- Weiss, J., Scherze, I., & Muschiolik, G. (2005). Polysaccharide gel with multiple emulsion. *Food Hydrocolloids*, 19, 605–661.
- Winter, H. H. (1997). Analysis of dynamic mechanical data: inversion into a relaxation time spectrum and consistency check. *Journal of Non-Newtonian Fluid Mechanics*, 68, 225–239.
- Winter, H. H., & Mours, M. (2006). The cyber infrastructure initiative for rheology. *Rheologica Acta*, 45, 331–338.
- Xiong, Y. L., Aguilera, J. M., & Kinsella, J. E. (1991). Emulsified milk fat effects on rheology of acid-induced milk gels. *Journal of Food Science*, 56(4), 920–925.
- Yamamoto, F., & Cunha, R. L. (2007). Acid gelation of gellan: effect of final pH and heat treatment conditions. *Carbohydrate Polymer*, 68, 517–527.