



## Compression pressure-induced synergy in xanthan and locust bean gum hydrogels. Effect in drug delivery

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### ABSTRACT

The study assesses the potential of combining xanthan gum and locust bean gum to create controlled-release hydrogels and also examines how compressing the polysaccharide blends affects their behavior. The research involved evaluating the rheological properties of aqueous dispersion of these gums and their mixtures using a rotational rheometer. Xanthan gum displayed a pseudoplastic Bingham behavior, while locust bean gum exhibited lower viscosity and shear thinning behavior. Gum blends showed an intermediate behavior with varying yield shear stress based on the gum proportions in the mixture. The flow index ( $n$ ) and the consistency index ( $K$ ) were calculated using the Power Law model (Ostwald de Waele relationship). The values of  $n$  are between 0.6 (Xanthan gum) and 0.1 (locust bean gum), with the blends showing intermediate values. These values are characteristic of shear-thinning systems and show a higher deviation of Xanthan gum from Newtonian behavior than LBG. Additionally, the  $k$  values indicate higher consistency for the Xanthan dispersions and similar behavior for the mixtures of the two polysaccharides, regardless of their proportion.

Precompression at 433.6 MPa for 5 min reduced the dispersion viscosity of xanthan gum by 50% at a shear rate of  $2\text{ s}^{-1}$ , decreasing it from 70 Pa s to 34 Pa s, while having a minimal impact on the rheological behavior of locust bean gum, which changed from 0.18 Pa s to 0.23 Pa s at a shear rate of  $2\text{ s}^{-1}$ . Temperature also decreased the xanthan gum dispersion viscosity. Oscillatory rheology tests highlighted differences between xanthan gum and locust bean gum dispersions. Xanthan gum formed a durable three-dimensional network with bonds, while locust bean gum exhibited a network with a shorter shelf life. Precompression significantly altered the rheological behavior of the gum blends, increasing the storage and loss moduli.

Drug diffusion tests developed in Franz-Chien cells showed no significant differences in diffusion profiles between gums and gum mixtures at a concentration of 0.5% w/v, indicating similar microviscosity to water at this concentration. Regarding drug release from xerogels prepared by compression, the hydrogels displayed sustained release, with release rates dependent on pH and the specific drug used. Locust bean gum showed consistent release rates at both pH levels, while xanthan gum and xanthan gum-locust bean gum blends exhibited lower release rates at pH 6.8. Triamcinolone acetonide, one of the tested drugs, showed slow release regardless of pH and gel composition.

In conclusion, the study suggests that precompression and temperature can impact the rheological behavior and release properties of gum gels, making them valuable parameters for controlling the release properties of these hydrogels.

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## 1. Introduction

Polysaccharides are very suitable components of hydrogels intended for pharmaceutical and biomedical applications, pharmaceutical or the food industry [1]. Most polysaccharides are non-toxic, biocompatible and biodegradable and exhibit interesting rheological properties in aqueous medium. Certain combinations of polysaccharides display synergistic interactions, leading to enhanced rheological properties, swelling capabilities, and drug release performance. One of the first examples of such synergistic interactions was described for xanthan gum (XAG) and galactomannans in 1971 by Rocks [2], showing that the mixture was able to form temperature-reversible gels. Since then, this interaction and the inherent viscoelastic properties of the mixtures have been widely studied [3–9]. XAG (Fig. 1) is a high molecular weight ( $10^6$  Da) polysaccharide produced by plant pathogenic bacteria of the genus *Xanthomonas* spp. [10].

The polymer backbone consists of four  $\beta$ -D-glucose units linked at positions 1 and 4, so its structure is identical to cellulose. Three-saccharide side chains on alternating anhydroglucose units distinguish xanthan from cellulose. Each side chain comprises a glucuronic acid residue between two mannose units. The resulting stiff polymer chain may exist in solution as a single, double or triple helix that interacts with other XAG molecules to form complex, loosely bound networks. The secondary structure of XAG can undergo a single transition from a disorganized and random configuration, with the side chains oriented away from the backbone chain to a rigid helicoidal structure where side chains fold along the backbone. This transition depends on the temperature and ionic strength of the solution; the organized structure is stable at low temperatures and high ionic strength. The intra- and intermolecular associations of XAG chains make the polysaccharide useful as a thickening and stabilizing agent in food, cosmetic, and pharmaceutical products, among other industrial applications. At room temperature (double-helix ordered structure), the intramolecular association involves hydrogen bonding between the hydroxyl group at C-3 and the hemiacetal oxygen atom of the D-glucosyl residues, and van der Waals interaction between the methyl group of the acetyl residue and the hemiacetal oxygen atom of the D-glucosyl residue. Changes in the secondary structure induced by an increase in temperature (random coil conformation) lead to significant modification of the rheological properties of xanthan aqueous solutions due to the breakdown of the intramolecular association and the formation of new interactions through pyruvate methyl groups in the side chains.

Galactomannans, such as Locust bean gum (LBG, Fig. 1), can

synergistically interact with the helices of other biopolymers, particularly XAG, powering their gelling ability [10–13]. Due to these interactions, the combination of these polysaccharides has been proposed for different areas, including drug development [12,14], biotechnology [15,16] or in the food industry [17,18].

LBG is a naturally occurring plant material obtained from seeds of *Ceratonia siliqua*, which consists of D-galactose and D-mannose units combined through glycosidic linkages, with a total molecular weight of 310,000 Da. Binary mixtures of xanthan and galactomannan may gel under conditions where the individual components are non-gelling. The synergistic gelation occurs by direct interaction between the two polymers, in which the D-mannose side chain of xanthan and the hydroxyl group at C-2 of the galactomannans backbone are involved. This results in the insertion of the xanthan molecule into the unsubstituted segments of the galactomannan backbone. The effect and intensity of synergistic interactions depend on factors related to the type of xanthan (ratio of acetyl substituent on the chains [18]), galactomannan (branching degree [19]) or the temperature (xanthan conformation [18]). Thus, by controlling the polysaccharide composition, their relative proportion and the conformation of xanthan chains by temperature treatments, the mechanical properties of the gels can be modulated [8].

In pharmaceutical and food industries, physically cross-linked hydrogels are particularly attractive as their preparation is relatively easy and avoid the use of chemical crosslinkers. Some chemical cross-linking molecules may be toxic [20–23] or affect the integrity and stability of the active molecules loaded in hydrogels. Consequently, it is necessary to remove excess of these toxic unreacted crosslinkers prior to application to the organism to avoid this toxicity. Among the various methods reported in the literature to obtain physically cross-linked hydrogels, compression at high pressure is particularly attractive [24–27]. This method consists of compressing a mixture of components using high-pressure to obtain xerogels. The high-pressure favors the physical interactions between the components during the compression step or later during the water absorption and swelling when the xerogels are in contact with an aqueous medium. It is possible to hypothesize that compression can be a suitable method to promote the entanglement of their chains, taking into account the nature of interactions between XAG and LBG and thus obtaining hydrogels with synergistic features. High pressures may induce conformational changes in molecules and thus may affect polysaccharide interactions.

The high consistency, water absorption capacity, and ability to control drug release of this type of hydrogel has the potential to be used for local drug administration through vaginal, buccal or rectal routes, as

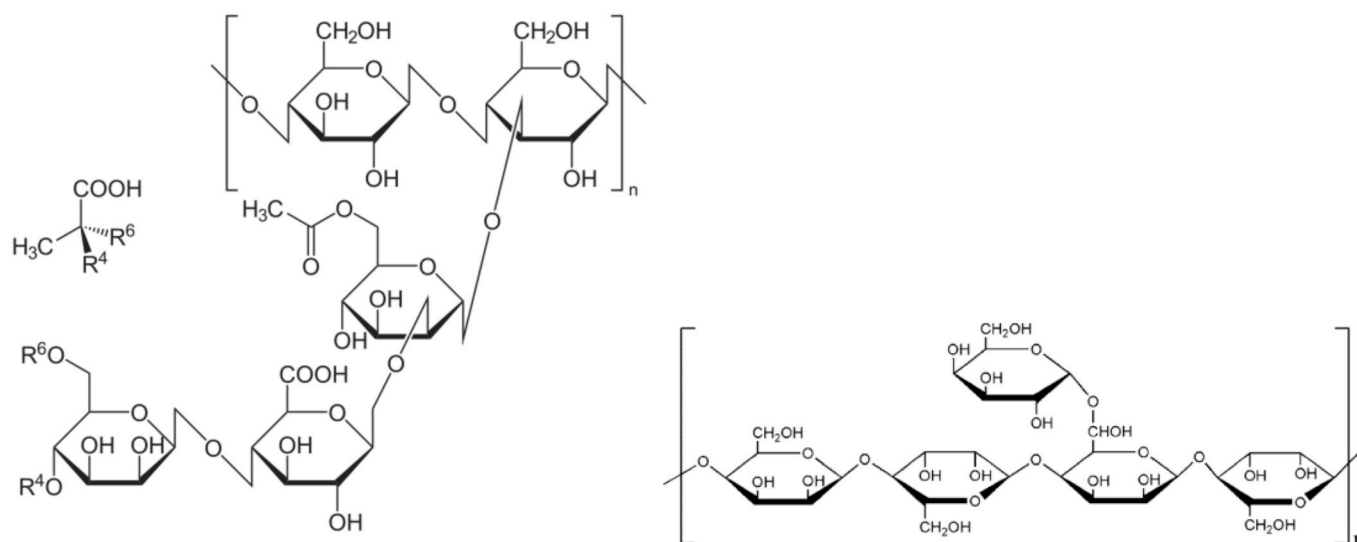


Fig. 1. XAG (left) and LBG(right) repeated units' representation.

well as for the development of controlled-release oral forms and colon-specific drug delivery.

In this study, the evaluation of how compression impacts the rheological behavior, structural characteristics, and interactions within aqueous solutions containing XAG, LBG, and their respective blend was assessed. Finally, the effect of the compression over diffusion of hydrophobic (triamcinolone acetonide) and hydrophilic (ciprofloxacin) drugs from the polysaccharide gels was also studied.

## 2. Materials and methods

### 2.1. Materials

Xanthan gum (XAG) and locust bean gum (LBG) were provided by Inagarbe S.L. (Padrón, Spain). Ciprofloxacin base (CPX) was from UQUIFA (Barcelona, Spain). Triamcinolone acetonide (TA) was acquired from Roig Farma (Terrassa, Spain). Potassium dihydrogen phosphate and sodium chloride were purchased from Panreac Química S.A. (Barcelona, Spain); dodecahydrate sodium dihydrogen phosphate and potassium chloride from Merck (Darmstadt, Germany). Ultrapure water was obtained by reverse osmosis (MilliQ, Millipore; Madrid, Spain).

### 2.2. Preparation of polysaccharide dispersions

Dispersions of XAG, LBG and their mixtures (Table 1) at a concentration of 0.5% w/w were prepared by their dispersion in water at room temperature or in boiling water. The dispersions were stirred for 24 h.

### 2.3. Preparation of xerogels

Xerogels of XAG, LBG and their mixtures were obtained by compressing 250 mg of polysaccharides in an IR-matrix pan fitted with a die of 12 mm in diameter, applying a force of 5 tons for 5 min using a hydraulic press Mega B-20 (Vizcaya, Spain). The obtained tablets were pulverized in a mortar and the polysaccharide dispersions were prepared from the powder as described above. Dispersions of XAG/LBG mixtures were prepared starting from either the polysaccharides compressed separately (5M5T) or from the mixtures compressed together (M5M5T).

### 2.4. Fourier-transformed infrared spectroscopy (FTIR)

Samples of XAG, compressed XAG, LBG, compressed LBG and compressed LBG:XAG 50%–50% blends were scanned from 400 to 4000  $\text{cm}^{-1}$ , with resolution of 4  $\text{cm}^{-1}$  using a FTIR GladiATR™ (Varian Pike Technologies).

### 2.5. Differential scanning calorimetry

The thermal properties of XAG, compressed XAG, LBG, compressed LBG and compressed LBG:XAG 50%–50% blends were registered using a TA Instruments® Q1000 SC/TGA/IR analyser (TA Instruments, Newcastle, USA). Samples were stabilized at  $-50\text{ }^{\circ}\text{C}$  and then heated to  $40\text{ }^{\circ}\text{C}$  at a rate of  $10\text{ }^{\circ}\text{C}/\text{min}$  under nitrogen atmosphere.

**Table 1**

Percentage of XAG and LBG in the polysaccharide mixtures used to prepare 0.5% w/w aqueous dispersions.

	Ratio in the polysaccharide blends (% w/w)						
XAG	100	75	60	50	40	25	0
LBG	0	25	40	50	60	75	100

### 2.6. Rheological studies

Rheological behavior of the dispersions was acquired using a rheometer AR1000-N (TA Instruments, UK) equipped with a Peltier plate and cone geometry (6 cm in diameter,  $2^{\circ}$ ). Oscillatory tests were carried out from 0.05 rad/s to 50 rad/s at 0.1 Pa and  $25\text{ }^{\circ}\text{C}$ . The storage ( $G'$ ) and loss ( $G''$ ) moduli were also recorded at a constant angular frequency of 1 rad/s and a force of 0.1 Pa while temperature increased from 20 to  $80\text{ }^{\circ}\text{C}$  at  $3\text{ }^{\circ}\text{C}/\text{min}$ . Creep and recovery tests were performed applying 0.1 Pa for 5 min and then recording the recovery for another 5 min. Flow tests were performed by applying a ramp of shear stress from 0.1 Pa to 10 Pa at  $25\text{ }^{\circ}\text{C}$ .

### 2.7. Drug diffusion tests

Dispersions of XAG, LBG or their mixtures at 0.5% w/v were prepared as described above but adding triamcinolone acetonide (20  $\mu\text{g}/\text{mL}$ ) or ciprofloxacin (50  $\mu\text{g}/\text{mL}$ ) to the aqueous medium. The dispersions were stored for 24 h before diffusion test using vertical Franz-Chien cells. The donor compartment with 1 mL of formulation was separated from the receptor compartment (filled with 7 ml of physiological saline solution NaCl 0.9%) using a dialysis membrane of 12000 Da MWCO. The diffusion area was  $0.7854\text{ cm}^2$ . At pre-established time intervals, samples of the receptor medium were taken for drug quantification and the volume was replaced with fresh medium. All tests were performed in triplicate at  $37\text{ }^{\circ}\text{C}$  and the receptor was stirring at 100 rpm. Diffusion coefficients,  $D_{app}$ , were estimated applying the Higuchi model for semi-solid systems.

$$\frac{Q}{A} = 2C_0 \sqrt{\frac{D_{app}t}{\pi}} \quad \text{Eq. (1)}$$

where  $Q/A$  represents the amount of drug released per unit of area at time  $t$ , and  $C_0$  the initial concentration of the drug in the vehicle.

Dispersions microviscosity were calculated using the Stokes-Einstein equation:

$$D_0 \cdot \eta_0 = D \cdot \eta \quad \text{Eq. (2)}$$

where  $D_0$  and  $\eta_0$  are the diffusion coefficient of the drug and the viscosities in absence of polysaccharides, and  $D$  and  $\eta$  the diffusion coefficient of the drug in polysaccharide dispersion and the microviscosity respectively.

### 2.8. Swelling properties of the xerogels

The xerogel were obtained by direct compression of 500 mg of XAG, LBG and their mixtures at 50% applying 5 tons for 5 min. The xerogels were immersed in 250 ml of hydrochloric buffer pH 1.2 and phosphate buffer pH 6.8 at  $37 \pm 0.5\text{ }^{\circ}\text{C}$ . At 2, 4, 6, 8, 10, 24, 30, 48 and 72 h the tablets were removed from the medium, the water excess was carefully dipped with paper, and then the diameter, height, and weight were recorded. The swelling degree was calculated as the ratio of the final volume to the initial volume. The water uptake expressed as grams of buffer absorbed per gram of dry polysaccharide was also determined. The test was performed in triplicate.

To identify the swelling mechanism, water uptake and swelling degree data was fitted to a model of Fickian diffusion of water molecules toward the xerogels using the next equation:

$$\frac{V_t}{V_0} = kt^{1/2} \text{ or } \frac{W_t}{W_0} = k't^{1/2} \quad \text{Eq. (2)}$$

Where  $V_t/V_0$  and  $W_t/W_0$  represent the swelling degree and water uptake at different times 't', and  $k$  and  $k'$  are constants dependent on the water diffusion coefficient in the xerogel.

Also, the evolution of the swelling, diffusion, and erosion front in

both buffers over time was investigated. To facilitate the detection of the different front methylene blue was dissolved into the buffers. Xerogels were immobilized between two sheets of glass so that the buffer only penetrated through the lateral layers. The evolution of each areas front was measured by taking photographs at different times and analyzing the fronts using imagePro Plus 6. o software (Media Cybernetic).

## 2.9. Release assay

Five mg of triamcinolone acetonide or 100 mg of ciprofloxacin were added to the polysaccharide/s to obtain hydrogels as previously described, maintaining the final weight constant in 500 mg. The release tests were carried out in a USP dissolution test apparatus 2 with paddles (Prolabo, Paris, France) connected through a peristaltic pump (HP 89092A) to a diode array spectrophotometer (HP 8452A; Palo Alto, EEUU). The release medium was 450 ml of hydrochloric buffer pH 1.2 for the first 2 h, and then the pH was increased to 6.8 by the addition of a small volume of phosphate buffer pH 8.0 and NaOH 2 M. In that way, transit in the gastrointestinal tract was mimicked for a gastric emptying time of 2 h and an intestinal transit time of 24 h. The medium was kept under stirring at 100 rpm and  $37 \pm 0.5$  °C. All experiments were carried out with six replicates of each formulation.

Drug release kinetics were fitted to the Higuchi model for thin cylindrical shapes:

$$\frac{M_t}{M_\infty} = Kt^{1/2}$$

Where  $M_t/M_\infty$  is the fraction of drug released at each time point  $t$ , and  $K$  represents the Higuchi release kinetic constant.

## 2.10. Statistical analysis

Release and swelling profiles were fitted to the most usual kinetics and the results obtained for the different formulations were compared applying multifactorial ANOVA.

## 3. Results and discussion

### 3.1. FTIR and DSC analysis

No significant changes were observed in the FTIR spectra or DSC curves (Fig. 2) of the polysaccharides after the compression process. Similarly, compression of the mixture of XAG and LBG also showed no significant changes compared to pure polysaccharides. The typical glass transition process of polysaccharides is evident in the DSC curves with glass transition temperature ( $T_g$ ) values of approximately  $-10$  to  $-12$  °C, but no changes were observed after compression.

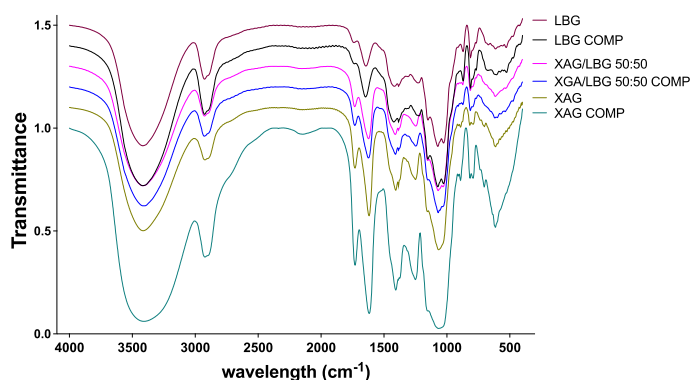
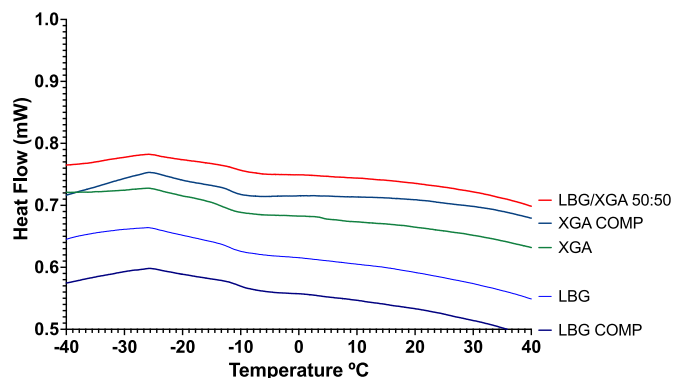


Fig. 2. DSC traces and FTIR spectra of XAG, LBG, and their 50/50 mixture before and after compression applying a force of 5 tons for 5 min in an IR-matrix pan equipped with a 12 mm diameter die.

### 3.2. Rheological properties

The aqueous dispersion of uncompressed XAG exhibited a Bingham pseudoplastic profile with a yield stress value of 1.3 Pa, (Fig. 3). At low shear rate values ( $<0.015$  s $^{-1}$ ), a slight increase in viscosity with the shear rate is observed. Above the yield stress, dispersions showed a shear-thinning behavior in which the viscosity gradually decreases with increasing shear rate. However, aqueous LBG dispersion had a remarkably lower viscosity with a typical shear thinning behavior (no yield stress value was observed). On the other hand, all the mixes show a Bingham pseudoplastic behavior with variable yield stress depending on the composition of the mixture. Only some differences in viscosity at small shear rate values between non-compressed XAG/LBG mixtures were observed (Fig. 3), the viscosity values at high shear rates were similar for all mixtures, showing lower viscosity than XAG but a very different behavior from that observed for LBG dispersion.

Viscosity curves at high shear rates (after yield stress) were fitted to the Power Law model (Ostwald de Waele relationship), the power law constant  $n$  (flow behavior index) and the consistency index  $K$  were calculated:

$$\eta = K\dot{\gamma}^{n-1} \quad \text{Eq. (2)}$$

where  $\eta$  is the apparent viscosity and  $\dot{\gamma}$  the shear rate. The equation properly fits the apparent viscosity after yield stress with  $R^2$  values  $>0.99$  and  $MRSE < 0.01$ . For un-compressed mixtures, the flow behavior index  $n$  values were  $<1$ , corresponding to shear-thinning systems (Fig. 3). XAG and its mixes show a higher deviation from Newtonian behavior than LBG, and  $n$  values were well correlated with proportion of XAG/LBG, according to the log-additive rule.

The consistency index  $K$  (Fig. 3) gives us an idea of the cohesiveness of the fluid so that the higher the  $K$ , the more viscous the dispersion. XAG shows the highest values of  $K$  and LBG the lowest. Interestingly, all the XAG/LBG mixtures show similar  $K$  values regardless of the proportion of XAG/LBG in the mix.

The dispersions obtained after compression of LBG hardly modifies the rheological behavior. However, a decrease in the viscosity of the pre-compressed XAG dispersion was observed, accompanied by a reduction in the consistency index ( $K$ ) from 3.62 to 2.96 Pa. (F test  $\alpha < 0.01$ ). Similar behavior was detected when XAG dispersions were heated at temperatures above 45 °C [28]. Viscosity of XAG dispersions depends on the secondary structure of the polysaccharide in solution. At low temperatures, the side chains were oriented away from the principal chain, establishing intramolecular interactions that result in an ordered single-stranded helix conformation and turn, in high viscosity dispersions. Over 45 °C, a breakdown of these intramolecular interactions occurs, enabling new interactions through the pyruvate methyl groups of side chains and leading to a helicoidally rigid structure where the side chains were folding along the backbone. This results in a decrease in the

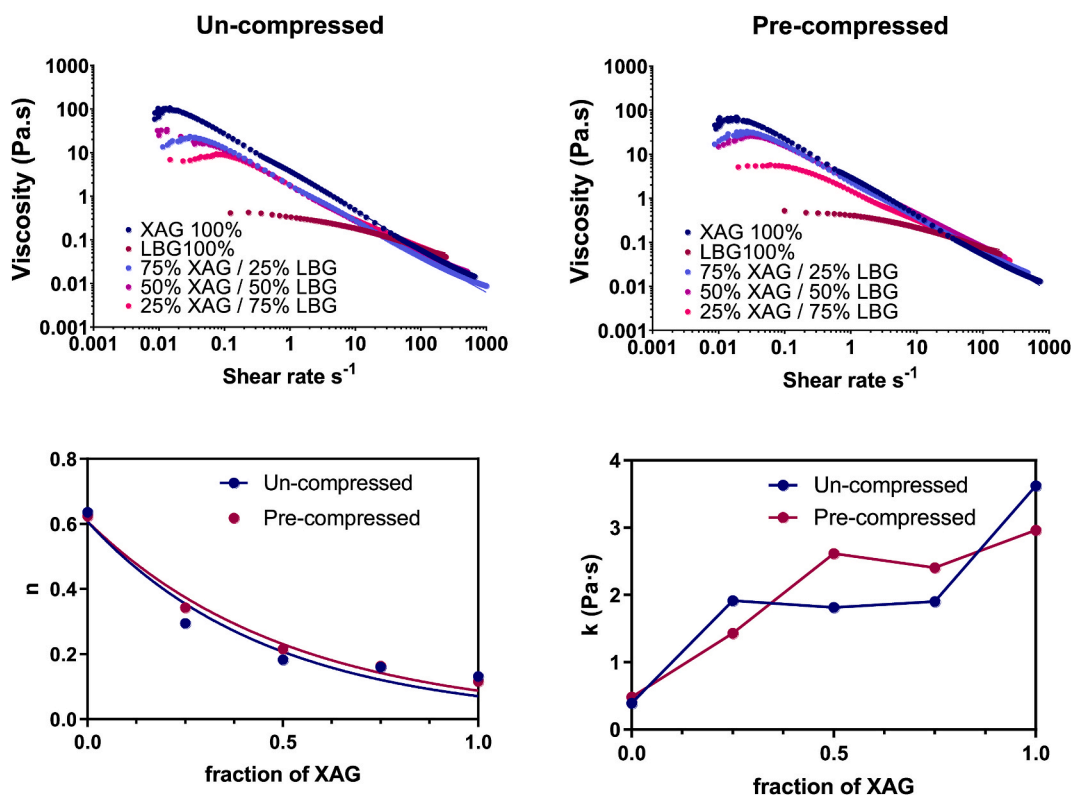


Fig. 3. Viscosity curves versus shear rate for a) un-compressed and b) pre-compressed polysaccharide dispersions, and ratio and variation of the c) flow behavior index “n” and d) the consistency index “K” of polysaccharide dispersions with the XAG/LBG ratio.

viscosity of the dispersion [8]. Similarly, the pre-compression step may have caused structural changes that persist after dispersion in water, which may be responsible for the lower viscosity.

In the case of XAG/LBG mixtures, a significant increase in viscosity values was observed, especially in those containing 50% and 75% XAG suggesting changes in the interaction between the two polysaccharides upon the pressure effect. These changes in behavior were also clearly shown in Fig. 3 if we compare the curves of percentage change in viscosity versus shear stress of un-compressed and pre-compressed polysaccharides calculated by using the equation proposed by Walker and Wells:

$$\% \text{ Change in viscosity} = \frac{\text{apparent viscosity} - \text{calculated mean viscosity}}{\text{calculated mean viscosity}} \times 100 \quad \text{Eq. (3)}$$

and the calculated mean viscosity  $\bar{\eta}_i$  was estimated by using the log

additive rule.

$$\log \bar{\eta}_i = X_a \log \eta_a + X_b \log \eta_b \quad \text{Eq. (4)}$$

As can be seen from the graphs in Fig. 4, there were changes in the percentage change in viscosity in both the un-compressed and pre-compressed mixtures. In the case of the un-compressed mixes, the changes were observed mainly for lower values of shear stress (<2). However, after compression of the mixtures of polysaccharides, this behavior was significantly modified, especially in the XAG/LBG 50%/50% samples.

Oscillatory rheology experiments were applied to investigate the changes in more detail. The oscillatory rheology tests also evidenced marked differences between XAG and LBG dispersions. LBG solely dispersion had lower values of G' than of G'' in the whole range of angular frequencies evaluated (Fig. 5). Only at the higher frequency tested (50 rad/s) a superposition of both moduli was observed,

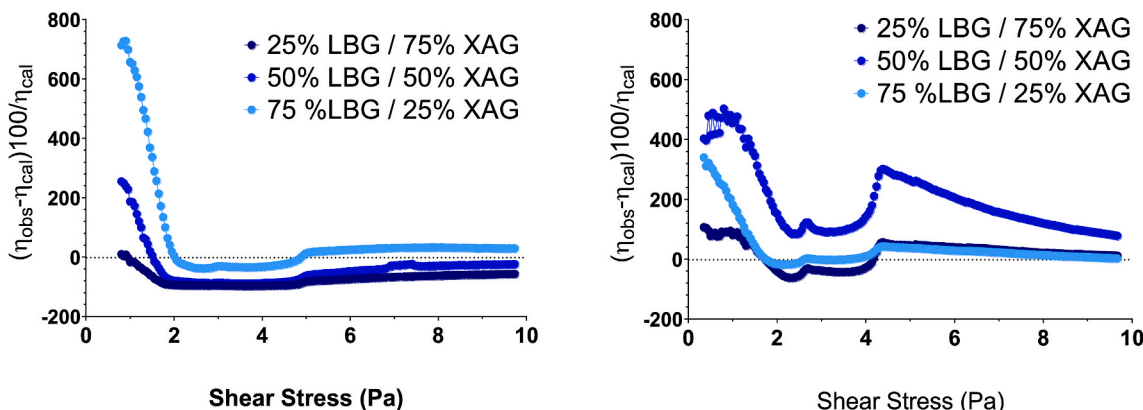


Fig. 4. Percentage change in viscosity of polysaccharide dispersions versus shear stress a) un-compressed samples, b) pre-compressed samples.

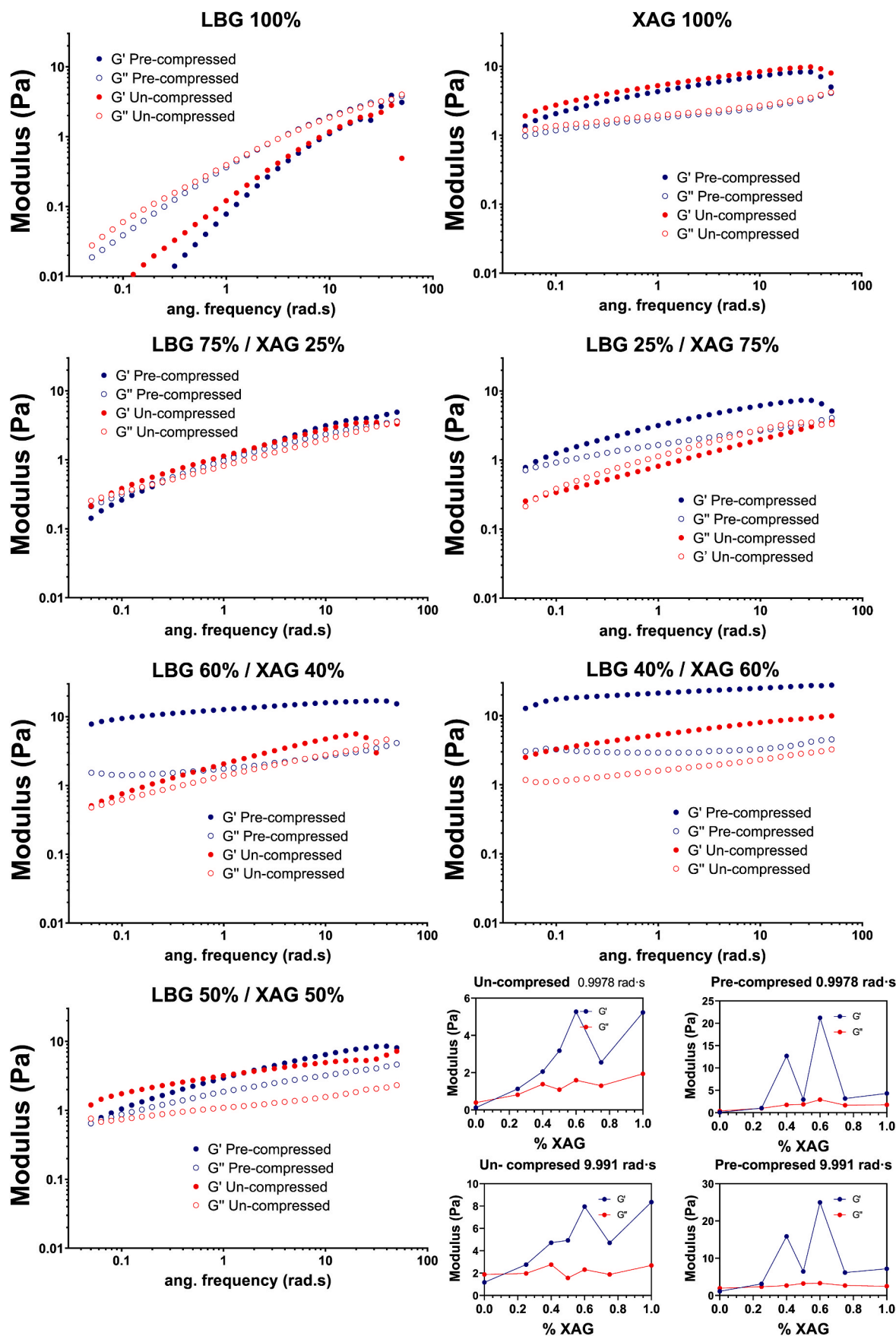


Fig. 5. Oscillatory rheology graph for pure polysaccharides and their mixtures and evolution of the storage  $G'$  and loss modulus  $G''$  in the mixes.

indicating that the entanglements among the chains have a short lifetime ( $1/50 = 0.02$  s). By contrast, XAG dispersions exhibited higher  $G'$  than  $G''$  values, disregarding the angular frequency, suggesting that the polymer chains form a three-dimensional network with long lifetime junctions ( $>20$  s). Once again, as the content in XAG increased, the rheograms of the mixtures progressively changed from the viscoelastic behavior shown by LBG to that exhibited by XAG.

Pre-compression of XAG or LBG significantly altered the rheological behavior of the blends, leading to an appreciable increase in  $G'$  and  $G''$  values (Fig. 4), obtaining even higher results than those recorded for the xanthan dispersion. Again, this suggests that changes in the conformation of polysaccharides caused by high-pressure compression may promote intermolecular interactions between both gums, resulting in a synergistic effect on the viscoelastic properties. To test whether the joint compression of both polysaccharides can modify this synergistic effect, mixtures of different proportions of XAG/LBG had compressed, and the rheograms had compared with those obtained with the mixes of the polysaccharides compressed separately (Fig. 6).

No significant differences were observed between the dispersion prepared with the polysaccharides compressed together and that obtained from un-compressed mixtures. However, higher values of both moduli had obtained with mixtures prepared by compressing each polysaccharide individually. The results suggest changes in rheological behavior probably due to conformational changes in the polysaccharide chains and not to the establishment of new intermolecular interactions between the different polysaccharides during the compression step, especially in those with a higher percentage of XAG. Dispersions of pre-compressed mixtures were prepared in cold and hot (boiling) water to gain an insight into the effect of the conformational changes and the storage and loss moduli recorded. To analyze this behavior, creep and recovery tests were carried out with the dispersions of XAG/LBG mixtures prepared in cold and hot water, applying a constant force of 0.1 Pa for 5 min and calculating the elastic recovery capacity (%) for a time of 5 min. Creep and recovery tests characterize the elastic behavior of the samples, and the greater the ability to recover the fluid more important the elastic component.

As shown in Fig. 7, an increase in the elastic recovery capacity had observed with the 40:60 XAG: LBG proportion. Nevertheless, no significant differences had detected between samples prepared using boiling water (100 °C), which have lower recovery capacity in mixtures with higher proportions of XAG and higher recovery for mixes with more LBG. Pre-compression stage significantly reduces the recovery capacity of the dispersions prepared with a high proportion of XAG, more pronounced than the effect of temperature. Nevertheless, recovery capacity had maintained and even increased in samples with 50 or 60% LBG. These results indicate that structural changes in the xanthan backbone produce a gel with lower elastic components, but the changes in LBG promote the formation of more elastic gels. Garcia-Ochoa et al. [3,28, 29] observed changes in the viscosity and XAG/LBG interactions when the LBG dissolution was carried out at different temperatures. At low temperatures, the regions with a high galactose/mannose ratio of LBG

were mainly dissolved. Nonetheless, as temperature increased, the chains of LBG that have a lower content in galactose content become dissolved. This low galactose content fraction has many smooth that can establish strong interactions with XAG molecules, especially when the proportion of LBG in the mixtures is high. The greater values in the elastic recovery capacity of XAG/LBG 60:40 dispersed in hot water must be due to the strong interactions between the polysaccharides at this temperature, so the increases in the elastic recovery capacity for pre-compressed mixtures suggest that high pressures also promote the establishment of stronger XAG-LBG interactions.

### 3.3. Drug diffusion tests

The diffusion coefficient in the hydrogels of the synthetic corticosteroid triamcinolone acetonide (MW: 434.51 g/mol, sparingly soluble in water at any pH) and the antimicrobial agent ciprofloxacin (MW: 331.34 g/mol, highly soluble in acid media) were selected to evaluate drug diffusion and microviscosity of hydrogels elaborated with mixtures of pre-compressed polysaccharides.

Fig. 8 shows the diffusion profiles of CPX and TA from the 0.5% w/w polysaccharides aqueous dispersions. CPX shows higher total drug diffused and diffusion coefficient values than TA in all formulations (Table 2), probably due to its smaller molecular size and higher hydrophilicity. One Way ANOVA and Tukey multiple comparison test of the total drug permeated at the end of the experiment was made. In the case of CPX, variations were found between XAG and LBG hydrogels ( $\alpha < 0.01$ ), exhibiting an intermediate behavior when combined. No differences in release were observed between the different XAG/LBG mixtures ( $\alpha$  n. s.). Nonetheless, there were no differences in the TA release between the two polysaccharides and their respective 50% XAG:50% LBG blends ( $\alpha$  n. s.). The diffusion coefficient values are very similar to those obtained by Stewart in 1996 [30] in water for ciprofloxacin ( $6.87 \times 10^{-6}$  cm<sup>2</sup>/s).

The microviscosity determination of the 0.5% w/w polysaccharide hydrogel dispersion shows that all the mixtures had similar microviscosities in concordance with the drug diffusion values. These values were only slightly higher than the viscosity of water at 37 °C (0.7191 mPa·s).

Xerogels from XAG, LBG and their mixtures were obtained to evaluate the potential of these hydrogel mixtures for controlled drug release. Also, their water uptake, swelling and drug release properties were characterized. After compression, xerogels had a white and bright aspect. Once immersed in buffer medium, the discs began to uptake water and swell, reaching equilibrium in 24 h. Fig. 9 shows the evolution of the hydrogel water uptake and swelling ratio versus time.

As it can be seen in Fig. 10, more prolonged time in the buffer medium caused the discs to dissolve/erode. The swelling was notably higher at pH 6.8 than at pH 1.2, as expected from the content in acid groups of the XAG chains. At pH 6.8, they become ionized, and the chains expand. In the case of LBG gels, the swelling was similar at both pHs in the first 10 h, but then, it rapidly increased at pH 6.8.

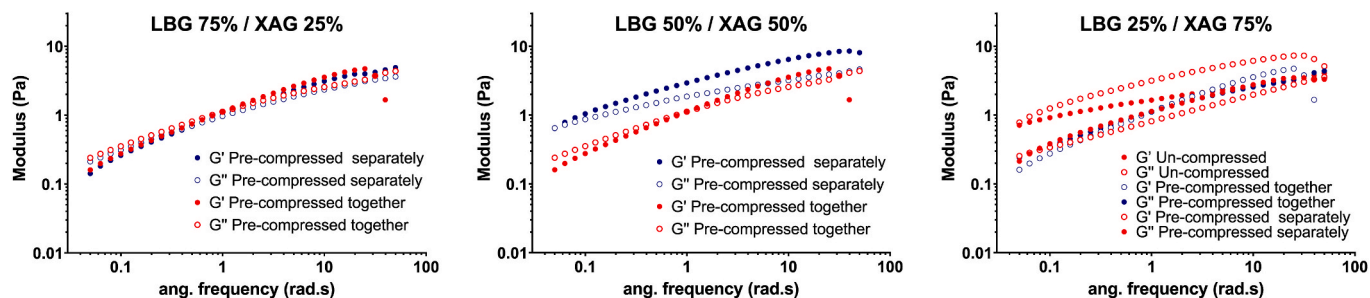


Fig. 6. Comparison of the storage  $G'$  and loss module  $G''$  between un-compressed mixes and mixes prepared with polysaccharides pre-compressed separately or together.

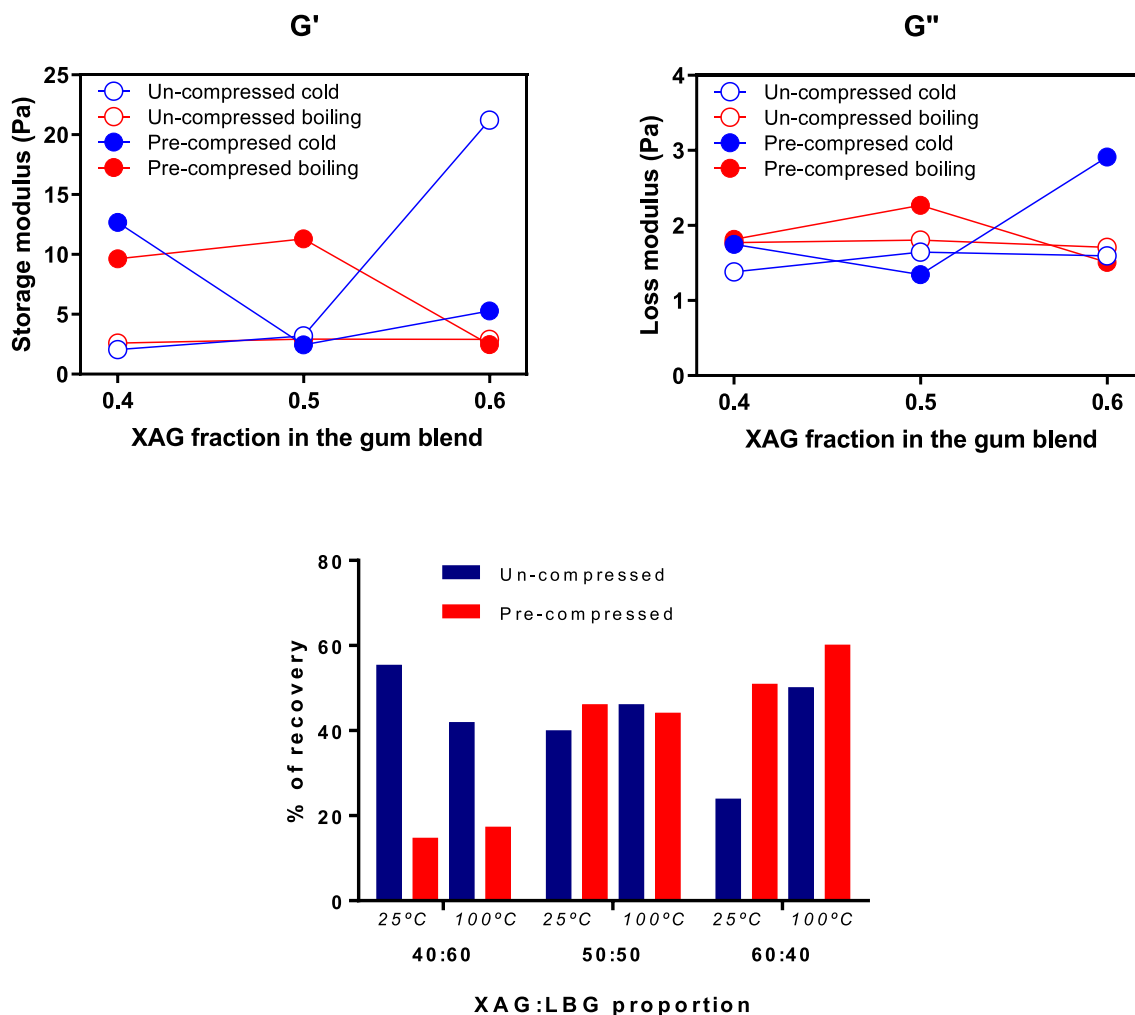


Fig. 7. Storage  $G'$  and loss  $G''$  modulus (angular frequency of 1 rad/s) and elastic recovery of polysaccharide dispersions (0.5% w/w) prepared with mixtures of gum with and without compression stage in cold and boiling water.

In all cases, the Higuchi equation was well-fitted for the water uptake, indicating that diffusion plays a principal role in the process (Table 3).

Fig. 11 shows the CPX and TA release profiles from the xerogels in simulated gastric fluid pH 1.2 (2 h) and intestinal fluid pH 6.8. Although the sustained release was observed in both media, differences were recorded depending on the drug considered. CPX release profiles fitted well to the diffusion release kinetics (Eq (3)) obtaining K values of 25.73, 18.92 and 20.94%/hr<sup>1/2</sup> in acidic media ( $R^2 > 0.996$ ) and 24.02, 10.20 and 10.38%/hr<sup>1/2</sup> ( $R^2 > 0.99$ ) from LBG, XAG and LBG:XAG 50:50 xerogels. LBG hydrogels provided the same release rate at both pHs, but XAG and LBG:XAG 50:50 xerogels showed a lower release rate at pH 6.8. CPX is protonated and highly soluble in acidic and basic medium but becomes a zwitterion at neutral pH. Zwitterion structures have poor solubility in water, neutral aqueous solutions, and polar solvents such as ethanol. Thus, the lower solubility, together with the possibility of the Zwitterion interaction with the acidic groups of XAG, can explain the decrease in the release rate at pH 6.8 and the change of the release kinetics to pseudo-zero order, despite the high swelling of the xerogels at this pH.

Xerogels containing TA released the drug very slowly, independently of the pH and gel composition. The release profiles showed pseudo-zero order kinetics with 20–25% released in 24 h. TA is practically insoluble in water (17 µg/ml), and as proven by the diffusion studies through the polysaccharide hydrogels, their diffusion coefficient through the fully hydrated hydrogels is smaller than that recorded for CPX.

The enzymes produced by anaerobic colonic flora can biodegrade polysaccharides. The gastrointestinal transit time for a monolithic pharmaceutical dosage is 5–10 h, depending on the feed state. During this time, the hydrogels released between 2 and 9% of the drug initially loaded; therefore, 91–98% can reach the colon. In this zone, the anaerobic colonic flora activity can cause the disintegration of the hydrated hydrogel, triggering the release of the remaining TA. Thus, these hydrogels could be suitable for large-intestine inflammatory disease treatment. More research is being carried out in our group to confirm this hypothesis.

#### 4. Conclusions

The article describes a study on the viscosity and rheological behavior of XAG and LBG dispersions in water. It had observed that XAG has a higher viscosity and exhibits pseudoplastic Bingham behavior, while LBG has a lower viscosity and shear thinning behavior. The mixes of both gums showed pseudoplastic Bingham behavior with varying values of yield shear stress as a function of mixture composition.

XAG and its mixes had observed to deviate more from Newtonian behavior than locust bean gum, and the flow behavior index values ( $n$ ) correlated with the ratio of XAG/LBG in the mixture. The consistency index ( $K$ ) showed that XAG had the most viscous dispersion while LBG had the lowest. However, all XAG/LBG mixes showed similar  $K$  values, regardless of the gum proportion in the mixture.

Precompression of XAG caused a decrease in the viscosity of the

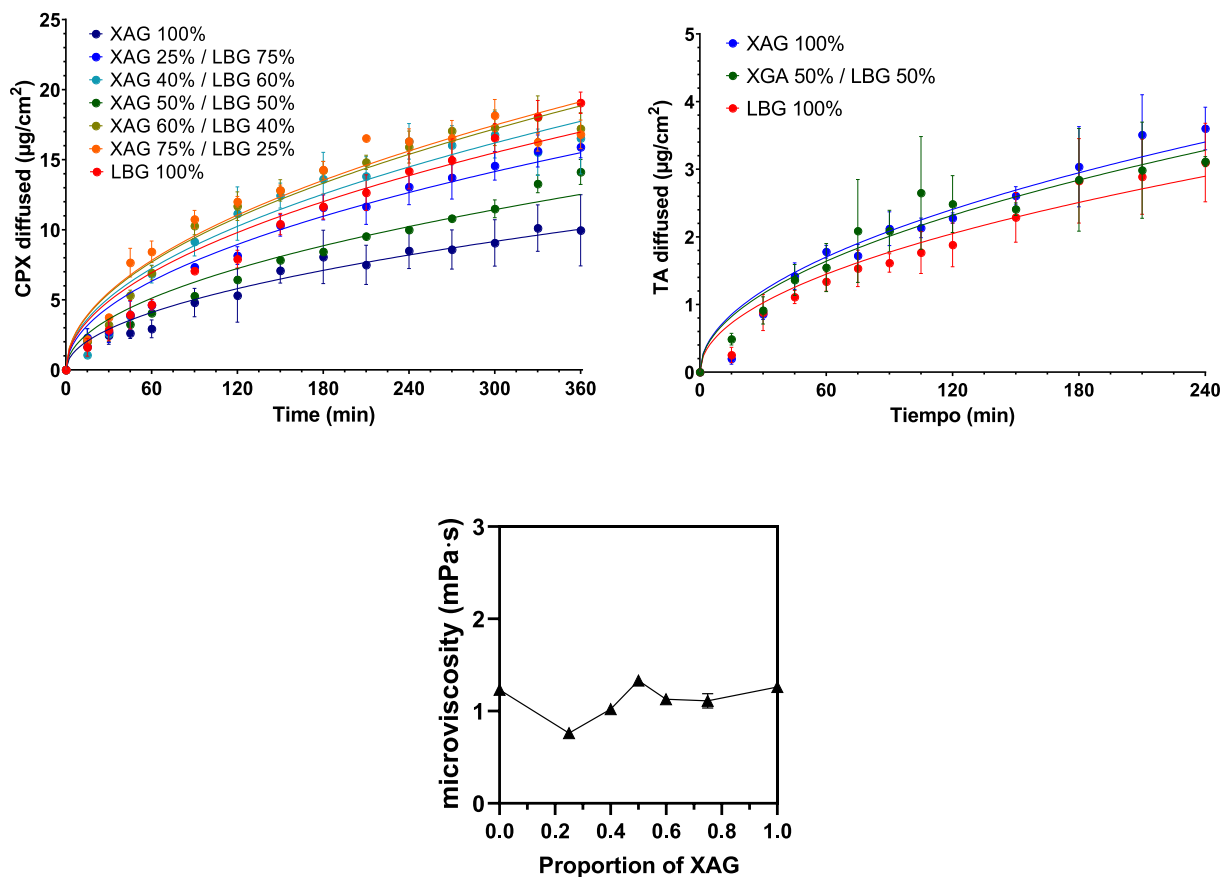


Fig. 8. Diffusion profiles of ciprofloxacin (top left) and triamcinolone acetoneide (top right). Evolution of the microviscosity calculated from ciprofloxacin diffusion coefficient in gels versus the proportion of XAG in the mixture.

Table 2  
Total drug diffused and Diffusion coefficient values of CPX and TA across 0.5% w/w polysaccharide dispersions.

XAG/LBG	D (cm <sup>2</sup> /s)		Total diffused drug (µg/cm <sup>2</sup> )	
	CPX	TA	CPX	TA
0/100	6.09 × 10 <sup>-6</sup>	0.121 × 10 <sup>-6</sup>	7.53 ± 2.02 <sup>a</sup>	2.83 ± 0.25 <sup>a</sup>
25/75	3.78 × 10 <sup>-6</sup>	–	12.49 ± 0.61	–
40/60	5.02 × 10 <sup>-6</sup>	–	12.98 ± 1.3	–
50/50	6.55 × 10 <sup>-6</sup>	0.117 × 10 <sup>-6</sup>	11.09 ± 0.70 <sup>a</sup>	2.44 ± 0.06 <sup>a</sup>
60/40	5.57 × 10 <sup>-6</sup>	–	13.52 ± 0.91	–
75/25	5.50 × 10 <sup>-6</sup>	–	13.20 ± 0.82	–
100/0	6.24 × 10 <sup>-6</sup>	0.160 × 10 <sup>-6</sup>	14.97 ± 0.60 <sup>a</sup>	2.43 ± 0.46 <sup>a</sup>

<sup>a</sup> Differences between TA and CPX for α < 0.05.

dispersions, whereas compression of LBG hardly altered their rheological behavior. Furthermore, the temperature were also observed to affect the viscosity of the XAG dispersions.

Oscillatory rheology tests revealed marked differences between the XAG and LBG dispersions. XAG formed a three-dimensional network with long-lasting bonds, whereas LBG exhibited a short-lasting network. Pre-compression of the gums significantly altered the rheological behavior of the mixes, increasing the values of the storage and loss moduli.

Drug diffusion tests revealed that the gel mixtures did not show significant differences in diffusion profiles, and the microviscosities of the hydrogel dispersions were similar to those of water. As for drug release, the sustained release had observed in the obtained gels, and the release rates varied as a function of pH and the drug used. LBG showed

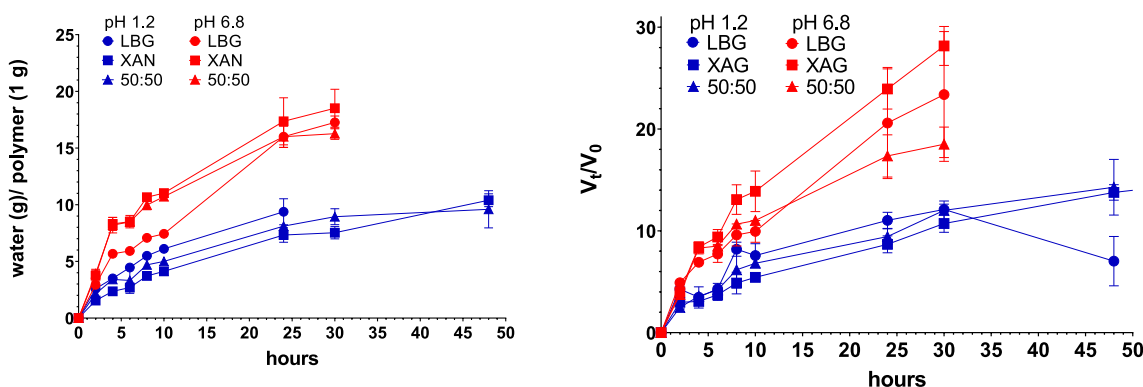


Fig. 9. Water uptake and swelling profiles (volume ratios) of compressed LBG and XAG mixtures in gastric and enteric simulated fluids.

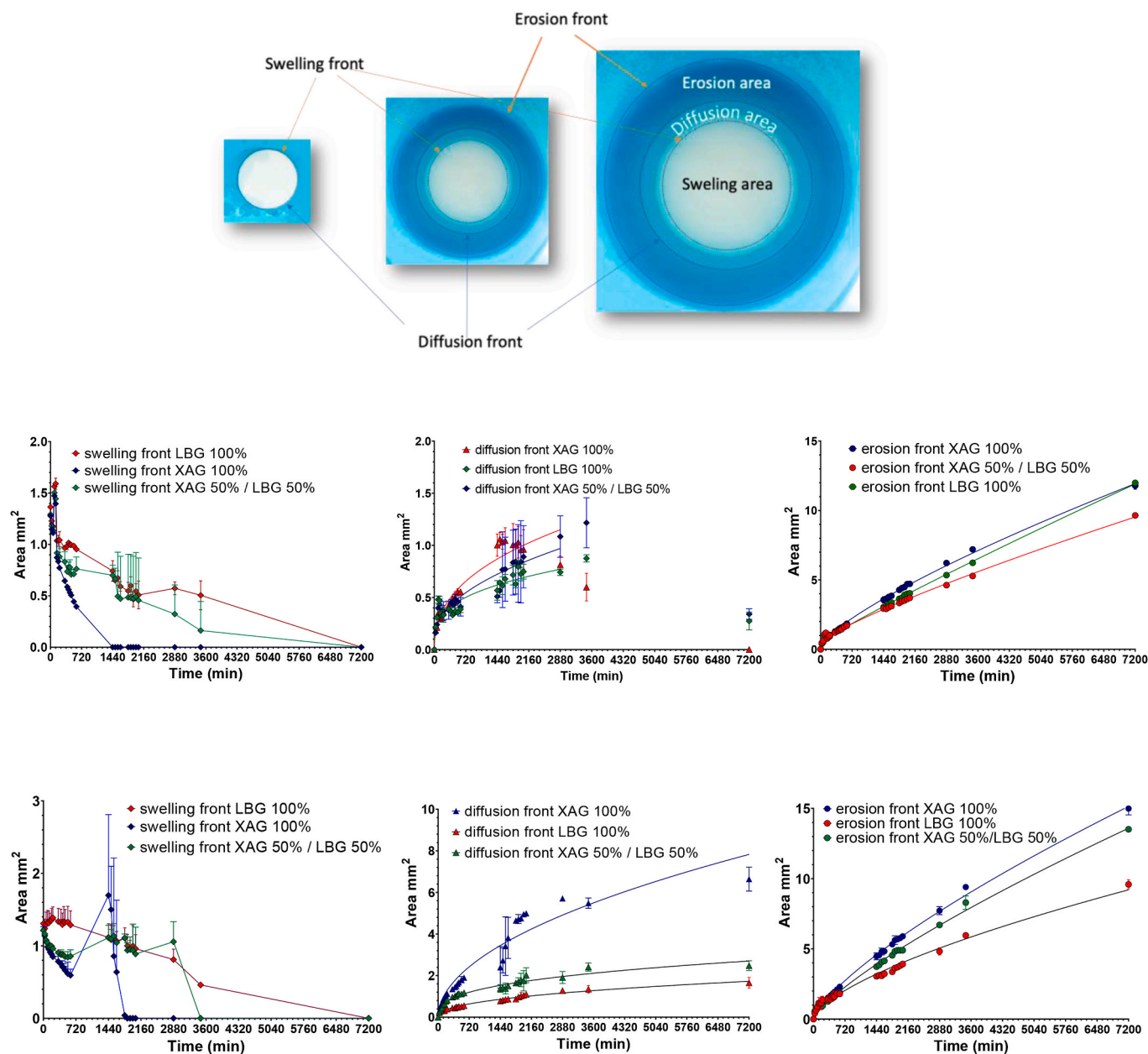


Fig. 10. Evolution in diffusion, erosion and swelling front (areas) vs time in simulated gastric (pH 1.2) and enteric (pH 6.8) fluids.

Table 3

Results of the fitting of water uptake and swelling to the Fickian water diffusion model (eq 2).

		pH 1.2			pH 6.8		
		LBG	XAG	50:50	LBG	XAG	50:50
Water uptake	$k^1$	1.937	1.536	1.486	3.299	3.47	3.055
	Std. Error	0.06612	0.04413	0.07477	0.1352	0.1399	0.1214
	$R^2$	0.9783 <sup>a</sup>	0.9798	0.9405	0.9644 <sup>a</sup>	0.9655	0.9664
$V_t/V_0$	$k$	2.28	1.987	2.127	4.339	5.292	3.47
	Std. Error	0.33	0.10	0.11	0.33	0.19	0.25
	$R^2$	0.8991	0.9910	0.9850	0.9763	0.9947	0.9745

<sup>a</sup> Time <24 h.

the same release rate at both pH levels, while XAG and mixtures of XAG and LBG showed a lower release rate at pH 6.8. The release of the drug triamcinolone acetonide was slow and independent of pH and gel composition.

In summary, the study concludes that pre-compression of gums and

temperature can be effective parameters for the modulation of swelling and gel release properties.

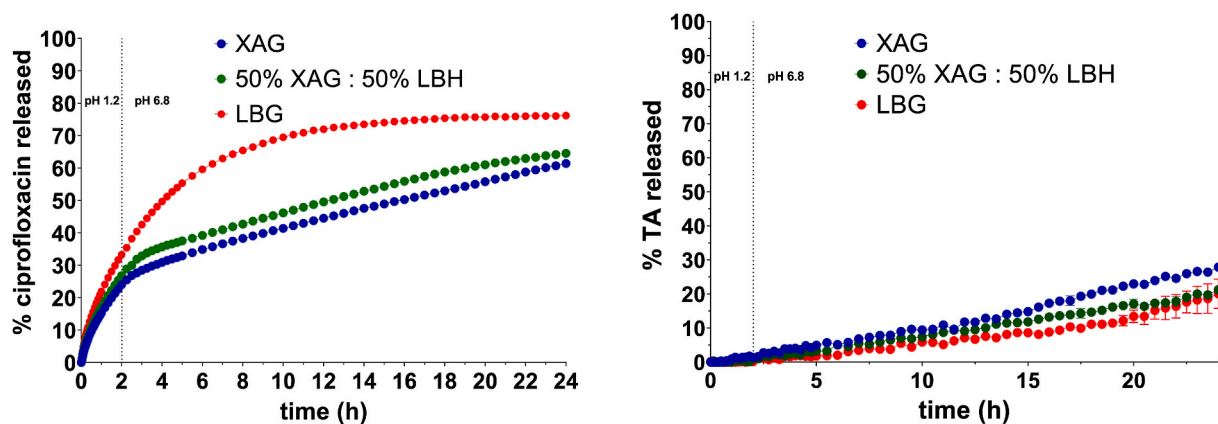


Fig. 11. Release kinetics of ciprofloxacin and triamcinolone acetone from polysaccharide hydrogels obtained by compression.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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