Gellan hydrogels: preparation, rheological characterization and application in encapsulation of curcumin

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Abstract

Hydrogels can be used to protect some labile active principles, as polyphenol-rich substances, that can be added to foods to prepare functional ones. Rheological properties of gels formed through the addition of calcium chloride to gellan solutions were studied. It can be concluded that preparation variables and not only formulation ones are determinant in rheological properties of the resulting gels, as they are not in an equilibrium state but they are continuously evolving during hours to stronger gels corresponding to a denser network. It could be related to the fact that local non-gelled domains are formed surrounded by a shell of gel where Ca^{2+} ions take some time to arrive. A minimum Ca^{2+}/gellan ratio (CG) is required to reach the gel point (GP), determined as the CG where the ratio loss modulus/elastic modulus (G”/G’) collapse for all frequencies. Calcium-induced external gelation of oil-in-water (O/W) emulsions where a curcumin-in-oil solution is the disperse phase and a watery solution of gellan is the continuous phase was used to prepare beads were curcumin is entrapped in order to prevent its degradation. Smaller droplet-sized emulsions were obtained with higher gellan concentrations, since a higher viscosity of the continuous phase allowed to reach the critical Capillary number Ca_C at lower radius of droplets. An encapsulation yield around 90 % was reached for gellan concentrations of 1 % w/v, and the resulting encapsulated curcumin presented around 6 times slower light degradation than free curcumin-in-oil solutions.

Keywords: Gellan, Calcium, Rheology, Curcumin, Beads, Hydrocolloid, Encapsulation

1. Introduction

Hydrocolloids, especially polysaccharides and some proteins, are extensively used in food industry, as they can provide specific and innovative textures [1-3], they can act as rheology modifiers and stabilize some structures

due to an increase in viscosity without substantially modifying other properties of the mixture such as flavour and colour [4]. Moreover, hydrocolloids can encapsulate and protect bioactive and/or insoluble ingredients that can be added to conventional foods in order to transform them into functional ones, which have currently an increasing demand [5]. Hydrocolloids could also find uses in targeted drug delivery systems [6, 7], in pharmaceutical and cosmetic industries [8, 9]. They can be used at concentrations below 1 % [10]. Polysaccharides form hydrogels through the addition of cations like K^+, Ca^{2+}, Mg^{2+}, that promote intermolecular junctions, acting as gelling agents. Other gelling agents used are oppositely charged polymers such as chitosan. These natural hydrogels are widely used because of their innate bioactivity and biocompatibility [11, 12]. Their thickening properties can find other medical uses, for example in the evaluation of the swallowing capacity in patients with dysphagia [13]. For this purpose, viscoelastic parameters must be accurately controlled since, for example, a less viscous than expected puree food would give a stronger swallowing problem, resulting in a wrong diagnosis of patients [14]. Therefore, it seems to be important to study if their cross-linking degree and, therefore, physicochemical properties depend not only on the composition (nature and concentration of hydrocolloid and counterion, hydrocolloid/counterion ratio, pH…), but also on the preparation process, in order to fix a protocol.

One of the polysaccharides used to form these hydrogels is gellan [15]. Gellan gum is a water-soluble anionic exopolysaccharide produced by Sphingomonas Elodea bacteria under aerobic fermentation of glucose [16]. It presents a tetrasaccharide repeating sequence of glucose (Glc), glucuronic acid (ClcA) and rhamnose (Rha) residues in a 2:1:1 ratio linked together to form the linear primary structure [D-Glc(β1→4)D-GlcA(β1→4)D-Glc(β1→4)L-Rha(α1→3)]$_n$ [17]. One of the advantages of gellan is that it is stable through a wide pH range [18, 19]. It forms gel at concentrations remarkably lower than other hydrocolloids such as carrageenan, alginate, pectin or gelatine [16]. This, coupled with its biocompatibility, high sorption capacity, hydrophilicity, low interfacial tension in contact with body fluids, good carrier properties, and high permeability of nutrients and metabolites make gellan an interesting scaffold material. Gellan gum also has the ability of improving the heat stability of other gelled products prone to melting when exposed to high ambient temperatures [19]. Divalent cations promote the gelation much better than monovalent cations [20, 21]. The effectiveness of cations to facilitate gelation decreases in the order $Ca^{2+} > Mg^{2+} > K^+ > Na^+$.

Encapsulation is a very useful method in protecting pharmaceutical ingredients, nutroceuticals and biologically active plant extracts [22]. Over the last few decades, encapsulation and controlled release technology have extensively been used in delivery of essential biologically active substances to specific portions of the gastrointestinal track [23]. Controlled and targeted release technology promotes more efficient utilization and less consumption of active agent, minimizes side effects and reduces frequency of administration. Gellan is one of the compounds that can be gelled and used in this type of delivery systems. Some recent publications report the encapsulation of curcumin with several methods that involve several polysaccharides combined with chitosan as gelling agent [24, 25].

Curcumin is an oil-soluble polyphenolic compound [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6- heptadiene-3,5-dione], extracted from the rhizomes of the herb Curcuma longa commonly known as turmeric [26, 27], with low intrinsic toxicity, extensively used as a spice, food preservative and colouring agent mostly in Asian countries. It contains polyphenolic compounds with strong antioxidant activity due to their ability to neutralize free radicals and oxidants [28]. Other therapeutic applications of curcumin are its antimicrobial, anticancer, antiinflammatory, antitumor, antioxidant and antiproliferative activities [22, 29-32]. It seems to be promising for the fight against cancer due to a certain ability to induce apoptosis in cancer cells [33] and appears to be useful against colon cancer [34, 35]. However, its applications are seriously limited because of low oral bioavailability due to its low water solubility and its physicochemical and biological instability [36-39]. Gellan gum is resistant to intestinal breakdown in the upper gastrointestinal track, it is digested by the colonic enzyme galactomannanase, and it has been shown in vitro that beads formed with gellan gum undergo initial surface erosion followed by rapid release of interior contents at pH and galactomannanase concentrations typically found in the colon [40, 41]. Research has proven that gellan beads significantly retard fast release of encapsulated material at pH 1.2 and achieve sustained release at pH 6.8 [23]. Therefore, as a hydrophobic substance, curcumin could be dissolved in an edible oil and encapsulated with gellan to be protected until be released. It could be a good way of supplying this and other similar substances to the human system because oils have structures compatible with biological membrane components.

The aim of this work was to study the rheological properties of gellan gels produced by chemical means with the use of calcium ions as gelling agent. The gel point, it is, the minimum Ca^{2+}/gellan ratio in order to obtain a gel, was determined by rheology, as well as the evolution of gels with time and the dependence of their rheological properties with preparation variables. Subsequently, gellan was used to encapsulate curcumin. For this, curcumin was dissolved in sunflower oil and the oil solution was emulsified in gellan solutions of several concentrations, which were then externally gelled dropping them into a Ca^{2+} bath. Beads formed and encapsulation yield were studied, as well as the reached protection of curcumin against degradation.

2. Materials and methods

2.1. Materials

Deacylated gellan gum was obtained from Solé i Graells (a food additives subministration Company). Calcium chloride anhydrous > 95 %, curcumin > 65 %, ethanol 96 % (v/v), and Tween 80, were obtained from Sigma-Aldrich. Commercial sunflower oil was used. MilliQ water was employed in the preparation of aqueous solutions. All chemicals were used as received with no further purification.

2.2. Preparation of stock solutions

Gellan solutions of different concentrations were prepared by dissolving the required quantity of polymer in MilliQ water under mild heating to 50 ºC and magnetic stirring for 30 minutes to dissolve the polymer since it

is insoluble in cold water [42]. These solutions were then allowed to cool at room temperature and thereafter kept in the refrigerator for 24 hours for complete hydration before use. Calcium chloride solutions at 1 % (w/v) were prepared for external gelation.

2.3. Preparation of gellan hydrogels

Hydrogels were prepared with an Ultra Turrax homogenizer model T.25 basic IKA-WERKE [43]. The gellan solutions were located in a beaker at room temperature, and different amounts of 1 % (w/v) calcium chloride solution were added in order to obtain the desired calcium-gellan ratio (CG) for each experiment. Immediately, homogenization in the range 11,000-22,000 rpm was applied for 3 minutes.

2.4. Rheological tests

The rheological properties of the hydrogels were studied using a rheometer HAAKE MARS (Modular Advanced Rheometer System) at a temperature of 25 ºC ± 0.1 ºC. A serrated plate-plate geometry with a diameter of 20 mm and 1 mm gap was used to avoid slippering. After loading, a resting time of 5 minutes was stablished before measurement in order to allow stress and temperature equilibration.

2.4.1. Oscillatory frequency sweep tests

Frequency sweep measurements at a fixed amplitude of shear stress were carried out in the frequency range of 0.01–10 Hz, at a controlled temperature of 25 ºC ± 0.1 ºC. Amplitude of stress applied was chosen in order to work within the linear viscoelastic region (LVR). To ensure that these conditions were met, preliminary stress sweep tests were made at a frequency of 1 Hz to choose a stress amplitude inside the LVR range, i.e., small enough for not modifying microstructure and, therefore, obtaining viscoelastic functions independent of imposed stress amplitude. Once analyzed the results obtained in the preliminary stress sweep tests, a fixed stress amplitude of 3 Pa was chosen for the frequency sweep measurements.

2.4.2. Steady state viscosity

The stationary viscosity of samples was measured for different shear rates in the range 0.01-100 s^{-1} at a controlled temperature of 25 ºC ± 0.1 ºC. Protocol was programmed in order to fix a shear rate and monitorize the viscosity *vs*. time, in order to take the viscosity value only when it remained constant with time. When a constant value of viscosity was reached, it was registered and subsequently a new shear rate was stablished until new steady state. It was done in all the range of shear rates stablished.

2.5. Determination of solubility of curcumin

The solubility of curcumin in oil and in ethanol was determined by preparing mixtures of different concentrations placing the solvent in a beaker and adding curcumin. Mixing was done with a magnetic stirrer

for 60 minutes at room temperature. After that, samples were centrifuged at 1300 rpm for 10 minutes in order to identify the existence of a precipitate.

2.6. Preparation of stock solutions for bead formation

Gellan stock solutions for the formation of beads were prepared as described in section 2.2 but with the addition of 1 % (w/v) Tween 80 to stabilize the O/W emulsions that would be prepared later dispersing in the curcumin-in-oil solutions. Curcumin-sunflower stock solutions of 0.1 mg/mL were prepared and protected from sunlight by keeping in darkness.

2.7. Preparation of emulsions

Prior to the preparation of the beads, oil-in-water emulsions were formed by emulsifying the curcumin-containing sunflower oil in the gellan solutions. 90 mL of the gellan solution were measured and transferred to a beaker. The beaker content was homogenized with the Ultra Turrax at a rate of 11,000 rpm, and while homogenizing 10 mL of curcumin-containing oil were added using a syringe. All homogenization process was done at room temperature.

2.8. Light back scattering measurements

Turbiscan MA 2000 was used to indirectly measure stability. A tube is vertically located in the equipment and a light of λ = 850 nm is emitted towards the tube. The system has two optical detectors, one of them detects the transmitted light (% T) and the other one detects the back scattered light at an angle of 135º (% BS). Both transmission and backscattering are measured along the tube. In a dispersed system, transmission and backscattering are related to concentration and size of droplets/particles. Therefore, changes in them are an indirect measure of destabilization process. For the emulsions studied, changes in % BS were chosen, as values of % BS were higher than % T and sensitivity was higher. Measurements were done from the bottom to the top of the tube. The % BS mean value from 1-1,7 cm from bottom was plotted *vs*. time in order to see changes which indicated destabilization.

2.9. Preparation of curcumin beads

The beads were prepared by dropwise addition of the bubble-free emulsion in a calcium chloride solution 1 % (w/v) using a 500 µL Handystep electronic pipette. The medium was continuously stirred during bead formation with a magnetic stirrer at 200 rpm to prevent aggregation of beads. The distance between the syringe and the surface of the calcium chloride solution was kept constant about 15 cm above the surface of the calcium chloride solution to enable formation of uniform and reproducible beads. The resulting beads were allowed to cure for 5 minutes in the calcium chloride solution. They were then separated by filtration with a sieve and rinsed three times with MilliQ water to remove any free calcium ions from the surface of the beads.

2.10. Confocal microscopy

Confocal microscopy of the emulsions was done to evaluate the nature of the droplets, assure that curcumin was located inside, and also to correlate the effect of gellan concentration on the size distribution of the oil droplets. Leica TCS SP2 confocal microscope equipped with a 488 nm Argon laser line and a 63X 1.32NA objective and DIC (Differential Interference Contrast) was used. There was no need for labelling the samples with a fluorescent material because curcumin is fluorescent.

2.11. Encapsulation yield

In order to determine the encapsulated curcumin in beads, it was extracted with ethanol and ethanol solutions subsequently analysed by spectrophotometry. To establish an appropriate time suitable for complete extraction, 500 beads were counted, weighted and then mixed with 100 mL ethanol 96 % (v/v) under continuous stirring, and absorbance of aliquots was measured along time until constant value. The absorbance was determined using a Perkin Elmer UV/VIS spectrophotometer at λ = 425 nm.

For measurement of remaining curcumin in oil solutions, 5 mL of solution were mixed with ethanol and ethanol was separated and subsequently analyzed by spectrophotometry.

A standard curve for curcumin was constructed preparing a standard solution of curcumin in ethanol and subsequent dilutions. Ethanol that had been in contact with empty beads was used as a blank.

3. Results and Discussion

3.1. Effect of resting time after gel preparation

Stock solutions prepared one day before were used in order to obtain the hydrogels. Calcium chloride solutions were added to the gellan solutions and homogenized with Ultraturrax for 3 minutes at 11,000 rpm as described in section 2.3. Gels prepared visually evolved with time to more viscous and elastic ones. Viscoelasticity of these samples was therefore analized at several resting times after preparation by oscillatory frequency sweep tests.

Figure 1 (a) corresponds to a calcium-gellan ratio (CG) of 0.0036 g Ca/g gellan at time 0 and 125 minutes after homogenization with calcium chloride at 11,000 rpm. As it can be observed, elastic modulus G' is higher than loss modulus G'' in all the range of frequencies studied for both cases, indicating that for this CG gelation occurs [13]. Hovewer, a well-developed gel usually presents the viscoelastic functions G' and G" nearly independent of frequency [44]. At time = 0 both G' and G" increase with frequency, indicating that the sample behaves as a weak gel [10]. Regarding the values, G' and G'' are much higher for 125 minutes than for 0 minutes, with a stronger increase with time in G' than in G" and a lower dependence of both parameters with frequency at 125 minutes, indicating a more developed gel than at t = 0 [45-46]. Therefore hydrogel structure

is reinforced with time in the range of time studied, indicating that intermolecular new junctions are appearing.

In Figure 1 (b) tan δ (or, what is the same, G''/G') is plotted as a function of time at different frequencies for CG = 0.0036 g Ca/g gellan. As it can be seen, tan δ decreases, showing once again an evolution to a more elastic behavior with time, indicating again that the elastic network is yet forming. It could be explained by the fact that, while gelation starts just at the moment when calcium ions are introduced into the gellan solution, as reflects Figure 1 (a), it starts to gellify locally. Then, interconnected lumps with a shell of gel are formed at the early contact Ca^{2+}-gellan points and, although system is mixed by homogenization, ions have to migrate across the shell of these lumps in order to reach the inside of non-gelled domains and gellify the whole bulk. It seems to indicate that although bulk gelation is apparently macroscopically homogeneous, it is not complete as microdomains exist where calcium ions have not yet arrived. Gelation continues along the time, and the system changes to a more developed gel. This behavior should be taken into account for uses where the control of rheological properties is essential, for example, for swallowing tests, where a protocol with a fixed time of ingestion after preparation of testing foods must be fixed [13].

Taking into account these results, for subsequent rheological determinations the freshly prepared gels were charged in the rheometer and a fixed resting time of 30 minutes was established before measuring for all samples in order to obtain comparable results.

3.2. Effect of homogenization speed

For the study of the homogenization speed the hydrogels were prepared as described in section 2.2 and 2.3, at a fixed calcium-gellan ratio of 0.0060 g Ca^{2+}/g gellan with a gellan concentration of 0.60 % w/v. These conditions are above the gel point as it will be shown in the *3.3* section, and were prepared under the same conditions except for homogenization speed. Values of elastic and loss moduli, G', G'', viscosity at a low shear rate (0.113 s^{-1}), μ, and shear factor, defined as SF = μ (0.113 s^{-1})/μ (1.275 s^{-1}) are listed in Table 1.

Table 1 Values of G', tan δ, τ_0, μ and SF (μ (0.113 s^{-1})/μ (1.275 s^{-1})) at different homogenization speeds. Gellan concentration = 0.60 % (w/v). CG = 0.0060. T = 25 ºC ± 0.1 ºC

Homogenization speed [rpm]	G' (0.1 Hz) [Pa]	Tan δ (0.1 Hz) [-]	μ (0.113 s^{-1}) [Pa·s]	SF [-]
11,000	70.99	0.10	80.01	7.96
13,000	76.10	0.09	92.40	9.28
19,000	84.19	0.08	127.90	11.90
22,000	88.34	0.07	133.30	13.82

In Table 1 it can be observed a mild elasticity increase (higher G' and lower tan δ) when homogenization speed is increased, as well as an increase of low shear viscosity and shear factor. The increase of shear factor is related to a higher shear-thinning behaviour, i.e. a decrease of viscosity when shear rate is increased as a result of a shear-induced disruption of structure. All these results point towards a slightly stronger gel formation when the homogenization speed used for preparation of gels is increased. This result agrees with that discussed in section *3.1*, i.e., the presence of microdomains where Ca^{2+} ions hardly arrive and, therefore, although gelation in bulk is reached, it is not in fact microscopically complete. When homogenization speed is higher, the lumps are smaller due to a higher shearing and the calcium ions are more homogeneously distributed, the non-gelled domains are smaller and the bulk gel stronger. Therefore, it can be concluded that shearing must be carefully controlled if reproducible results are required. For subsequent experiments, the homogenization speed was fixed at 11,000 rpm.

3.3. Determination of the gel point

The gelation process refers to the phenomenon which transforms a viscoelastic fluid into an elastic well-shaped solid (gel). The effect of Ca^{2+}/gellan w/w ratio (CG) in gelation and gel rheological properties was analyzed. The final gellan concentration in gels was fixed at 0.60 % (w/v). Steady state viscosity under shear and oscillatory experiments were carried out for several CG ratios from CG = 0 to CG = 0.03.

Figure 2 shows the steady state viscosity *vs.* shear rate for gellan in the absence of calcium and for several CGs. It can be observed an increase of viscosity with CG. All samples present shear thinning behavior, indicating a shear-induced disruption of structure. However, in the absence of calcium there is a range of shear rates where Newtonian behavior exists, indicating that the sample is able to flow without modification of structure when a low shear is applied. The same occurs, but in a range of lower shear rates, for a CG = 0.0015. This behavior is

typical of viscoelastic liquids, which can flow without disruption of structure when the shear is low enough. It is attributed to the presence of free disolved polymer molecules, or few Ca^{2+}-induced joined molecules but with junctions that do not extend to the whole bulk, as they can freely move. So, a network is not yet developed. At higher shear rates, these molecules or groups of molecules align in the direction of flow, some joining points can disentangle and shear-thinning occurs. At CG = 0.0015, viscosity is higher than at CG = 0 and the Newtonian range decreases, indicating that calcium ions induce some intermolecular junctions. However, they are not enough to gellify the sample. At CG = 0.0020 and above, the Newtonian zone has completely disappeared in the range studied, indicating that flow necessarily implies some kind of change or disruption of structure, a typical behavior of viscoelastic solids, which could indicate that the system is gelled and, therefore, a three-dimensional network exists in the whole bulk that needs to be disrupted for flowing. This network is stronger at higher CG due to the presence of more intermolecular junctions induced by the presence of more Ca^{2+}.

Viscoelastic functions G' and G" for some CG are shown in Figure 3. The sample without calcium was clearly predominantly viscous, with G" much higher than G'. For CG = 0.0015, G' and G" increased and got closer but being G" yet higher than G' for a range of low frequencies and increasing both with frequency, indicating yet a predominantly viscous behavior although with some elasticity, according to that discussed for steady state experiments. Behavior of sample with a CG ratio of 0.0020 drastically changed with G' clearly higher than G" and nearly independent of frequency, indicating that gelation point is located in some way between 0.0015 and 0.0020. Higher CG samples presented progressive higher values of moduli, with G' nearly independent on frequency, indicating a stronger gel formation (data not shown).

In order to accurately determinate the gel point, tanδ = G"/G' for several frequencies is plotted *vs* CG (g Ca^{2+}/g gellan) in Figure 4. According to [45, 46], at the gel point (GP) tanδ is independent of frequency and, therefore, all curves collapse, as GP is strictly dependent of the material and independent of the applied frequency. It can be seen that it happens at a CG around 0.0018 g Ca^{2+}/g gellan. So, this ratio is required in order to extend the intermolecular junctions to the whole bulk and form a well-developed three-dimensional network. The increase of G' and G" observed at higher CG is related to the formation of more intermolecular junctions, which produces a denser network, that behaves like a stronger gel.

Table 2 shows a summary of rheological parameters for all CG ratios tested. Tan δ was calculated for a frequency of 0.1 Hz. As it can be seen, G', as well as low shear rate viscosity, drastically increase when gelation occurs, in the range 0.0015<CG<0.0020, although it continuously increases with CG, as said before. Accordingly, tanδ strongly decreases around the gel point.

Table 2 Rheological parameters of the gels at different CG ratios

CG Ratio [Ca^{2+} [g]/gellan [g]]	G' (0.1 Hz) [Pa]	tanδ (0.1 Hz) [-]	μ (0.113s^{-1}) [Pa·s]
0	0.06	1.64	0.216
0.0015	0.27	1.54	1.25
0.0020	28.84	0.166	17.84
0.0030	33.86	0.118	53.67
0.0036	35.05	0.113	55.69
0.0045	70.99	0.092	68.98
0.0060	82.26	0.082	80.01
0.0301	405.90	0.069	79.55

3.4. Encapsulation of curcumin into gellan beads

3.4.1. Oil-in-water (O/W) emulsions of curcumin-in-sunflower/gellan

As curcumin is insoluble in water, in order to encapsulate it in gellan beads it is first required to dissolve curcumin in an oil and emulsify the oil in gellan solutions, previously to gelation. Curcumin solubility in sunflower oil was determined to be 0.1 mg/mL and in ethanol 96 % (v/v) was 2.5 mg/mL at room temperature. Curcumin was therefore about 25 folds more soluble in ethanol than in oil. The high solubility of curcumin in ethanol was exploited for extracting and quantifying curcumin from the beads.

Oil in water (O/W) emulsions of curcumin-oil/gellan at 10 % (v/v) of disperse phase (the oily phase) were prepared with the homogenizer as described in section 2.7 for gellan concentrations of 0.25 %, 0.50 % and 1.0 % (w/v). Confocal micrographs of emulsions were immediately taken and are shown in Figure 5.

From Figures 5 (a), (b) and (c), it can be observed that oil droplets formed are smaller when the gellan concentration is higher. Figures 5 (b) and (d) show equivalent images with Figure 5 (d) showing that curcumin, which is fluorescent, is all located inside the oil droplets. Low shear viscosities of gellan solutions are listed in Table 3, as well as mean droplet sizes. It is shown that when viscosity of continuous phase (gellan) increases, mean droplet size decreases. It can be related to the capillary number (Ca). Capillary number represents the relative effect of viscous forces versus surface tension acting across the interface between the two immiscibleliquids (Eq. 1), the first favouring deformation and breakage of droplets into smaller ones and the later minimizing the interface. Symbols τ and $\dot{\gamma}$ are the shear stress and shear rate applied, σ the surface tension, η_C the viscosity of the continuous phase and R the radius of droplets. A droplet elonges and breaks into two smaller ones as far as Ca reaches a critical value. As for all the emulsions a constant homogenization speed has been used (11,000 rpm), the shear applied is the same, as well as the surface tension. Therefore, when the

viscosity of the continuous phase increases due to an increase on gellan concentration, the radius of the droplets for reaching the critical value Ca_c decreases and, therefore, the mean droplet size of these emulsions is lower.

$$Ca = \frac{\tau}{\sigma/R} = \frac{\eta_C \dot{\gamma} R}{\sigma} \quad \textbf{(Eq. 1)}$$

Table 3 Viscosity, average droplet size and encapsulation yields at various gellan concentrations

Concentration of gellan in solution [% w/v]	Viscosity of gellan solutions before gelation [Pa·s]	Average size of oil droplets in O/W emulsions [μm]	Encapsulation yield after formation of beads [%]
0.25	0.32	77.5	30.9 ± 2.9
0.50	0.216	54.9	43.6 ± 3.1
1.00	1.42	37.4	89.5 ± 1.5

Figure 6 shows backscattering results for the emulsions formed with the three concentrations of gellan tested. The emulsions showed more stability at higher gellan concentrations, as the backscattering value changes were smaller with time. These results correlate with that obtained from confocal microscopy, where agglomeration of droplets can be observed at the lowest concentration of gellan (Figure 5 (a)).

At low gellan concentrations, the oil droplets can more easily move across the continuous phase, and agglomeration occurs, that eventually turns to coalescence forming larger oil droplets, decreasing the stability of the system. At higher concentrations, discrete, smaller and relatively monodisperse droplets are formed that move slower due to the high viscosity of the continuous phase, preventing agglomeration and subsequent coalescence, at least for a time, that can be enough for the ulterior gelation process.

3.4.2. Encapsulation of curcumin-in-oil in gellan beads through external gelation of emulsions

Emulsions were dropped in a 1 % w/v Ca^{2+} solution as stablished in section 2.9. Beads were maintained for 5 minutes in the Ca^{2+} bath for curing. It is important to fix a curing time since, according to section 3.1, gel evolves with time because ions need a time to migrate across the barrier of already formed gel. Here, the curing time is yet more determinant, as in external gelation of beads the ions are not mixed inside the droplets and they have to migrate from the external calcium chloride solution to the core of beads crossing the forming shell of gel. In fact, according to [28], where beads of alginate were prepared through external gelation with Ca^{2+}, it is expected a limitation of Ca^{2+} migration across the forming shell, obtaining beads with a more developed and denser network near the surface and a more open one around the core. These beads are, therefore, formed by an emulsion with a gelled continuous phase and a disperse phase cointaining the oil with the curcumin. Although,

as said, a homogeneous crosslinking of continuous phase is not expected, general trends observed in mechanical properties when bulk gelation was carried out are expected to be maintained. That is, an increase in viscoelastic parameters with gellan concentration is expected, if a minimum Ca^{2+} supply is guaranteed. The presence of disperse oil droplet could just slightly decrease these parameters, as, according to previous works, when the continuous phase behaves like a gel rheology is basically controlled by the continuous, solid-like phase [47, 48].

Figure 7 shows a picture of the beads formed. Once beads were formed, encapsulation yield was measured. Encapsulation yield at various gellan concentrations is illustrated in Table 3. It can be seen that around 90 % of the total added curcumin remained in the 1.0 % gellan beads after the process of dissolution, emulsification, gelation and washing. However, only 31 % remained when 0.25 % gellan was used. It is attributed to the fact that at higher concentrations of gellan, the stronger cross-linking of the polymer by Ca^{2+} ions formed more compact three dimensional structures which helped in entrapping the curcumin-in-oil droplets within the beads and prevented curcumin from degradation and leakage during washing. Along all the process samples were exposed to sunlight as flasks were transparent and, probably, that was enough for partial degradation when the gel was not developed enough. Some of the curcumin could also be lost during the washing of beads.

The remaining curcumin in beads prepared with 1.0 % gellan was measured after 5 days through the protocol described in section 2.11, and compared with values of remaining curcumin for curcumin-in-oil solutions obtained after the same time, in order to measure the protection against degradation that encapsulation offered. Both samples were exposed to sunlight at room temperature. Results obtained with the solution indicated that curcumin was almost completely degraded with a yield of only 5.2 % ± 0.7 % but, when the same amount of curcumin-in-oil solution was encapsulated in gellan beads and exposed to the same conditions, a yield of 30.2 % ± 3.8 % was observed. According to these results, encapsulation with gellan presented around 6 times slower photodegradation than curcumin-in-oil solutions. It is a promising advance although it requires further investigation as, probably, a combination of gellan with other component could improve results, as some studies point out with encapsulation of other substances [24, 25].

4. Conclusions

Calcium gelation-induced gellan hydrogel structures prepared through homogenization evolved with time to stronger gels. It was attributed to the time required for Ca^{2+} to reach all the gellan-junction points. Although a weak gel was quickly formed, it evolved to a denser, stronger gel, with time. On the other hand, higher homogenization speed produced stronger gels, with higher rheological functions, attributed to a more homogeneous distribution of Ca^{2+} in gellan, which promoted closer junction points. Therefore, it can be concluded that preparation variables must be accurately controlled in uses where the rheological properties are determinant.

The minimum Ca^{2+}/gellan ratio CG was determined to reach the gel point, GP, i.e. to extend the intermolecular

junctions to the whole bulk and form a three dimensional network with gel behavior. The GP was determined as the CG where tanδ = G"/G' is independent of frequency.

External gelation of curcumin-in-oil/gellan emulsions was carried out in order to entrap curcumin in gellan beads. Curcumin-in-oil droplets of O/W emulsions were smaller at higher concentrations of gellan, as the higher viscosity of the continuous phase promoted a higher Capillary number Ca and, as a result, a lower radius of droplets to reach the critical Ca_C. Curcumin was entrapped into the oil droplets. The curing time of beads inside the Ca^{2+} solution must to be controlled since ions require a time to migrate across the gelled shell of beads. A higher encapsulation yield, around 90 %, was reached at higher gellan concentrations, due to the formation of a more compact gel. Encapsulated curcumin presented around 6 times slower sunlight degradation than free curcumin-in-oil solutions, indicating that encapsulation offered some protection against oxidation.

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Figure Captions

Fig. 1 (a) G' and G" *vs*. frequency for a gel with gellan 0.60 % and calcium-gellan ratio of 0.0036 g Ca/g gellan at time 0 (G' triangles; G" circles) and 125 minutes (G' diamonds; G" squares); (b) Tan δ (G''/G') *vs.* time at different frequencies, 0.1 Hz (white diamonds); 0.215 Hz (white squares); 0.316 Hz (white triangles); 0.464 Hz

(black diamonds); 0.681 Hz (black squares); 1 Hz (black triangles). T = 25 ºC. Homogenization rate = 11,000 rpm

Fig. 2 Steady state viscosity *vs*. shear rate for gellan 0.60 % (w/v) and several CG ratios. T = 25 ºC. CG = 0 (circles), CG = 0.0015 (triangles), CG = 0.0020 (diamonds), CG = 0.0045 (squares). Homogenization rate = 11,000 rpm

Fig. 3 G' and G" moduli *vs*. frequency for gellan 0.60 % (w/v) and several CG ratios. T = 25ºC. G' (open symbols); G" (black symbol); CG = 0 (circles), CG = 0.0015 (triangles), CG = 0.0020 (diamonds). Homogenization rate = 11,000 rpm

Fig. 4 Tanδ = G"/G' *vs*. CG = g Ca^{2+}/g gellan for several frequencies. Gellan concentration = 0.60 % (w/v). T = 25 ºC. 0.1 Hz (circles), 0.215 Hz (open triangles), 0.464 Hz (crosses), 1 Hz (diamonds), 2.154 Hz (black triangles). Homogenization rate = 11,000 rpm

Fig. 5 Confocal micrographs of curcumin-in-oil/gellan emulsions 10 % v/v for (a) 0.25 % w/v gellan; (b) and (d) 0.50 % w/v gellan; (c) 1.00 % gellan. All the emulsions were prepared as described in section 2.7.

Fig. 6 Back scattering measurements for oil/gellan emulsions for a 0.25 % gellan (diamonds), 0.5 % gellan (squares) and 1 % gellan (triangles)

Fig. 7 Beads of gellan with curcumin-in-oil prepared by external gelation

Figure

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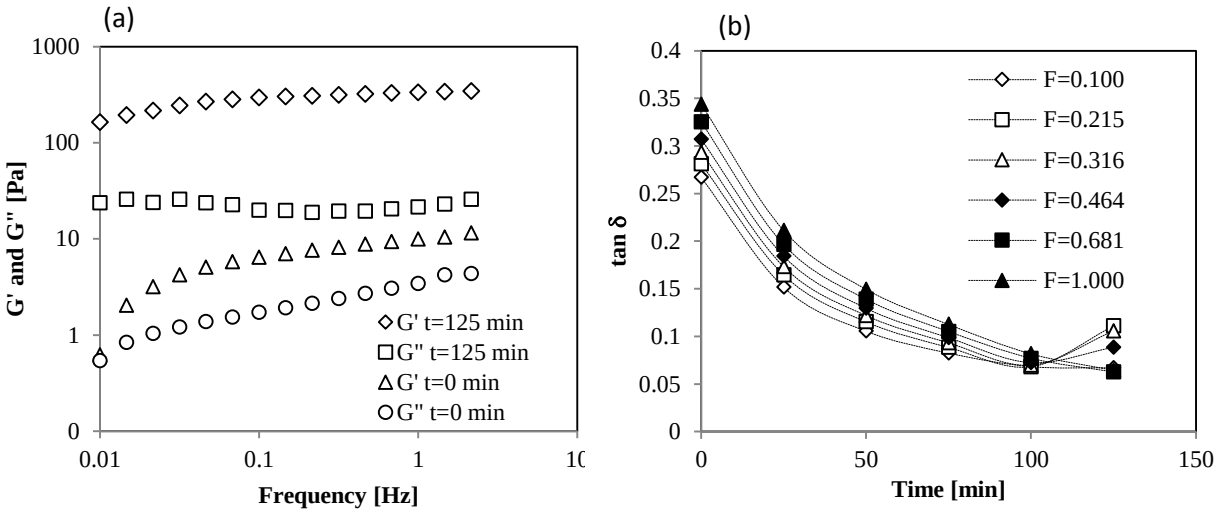

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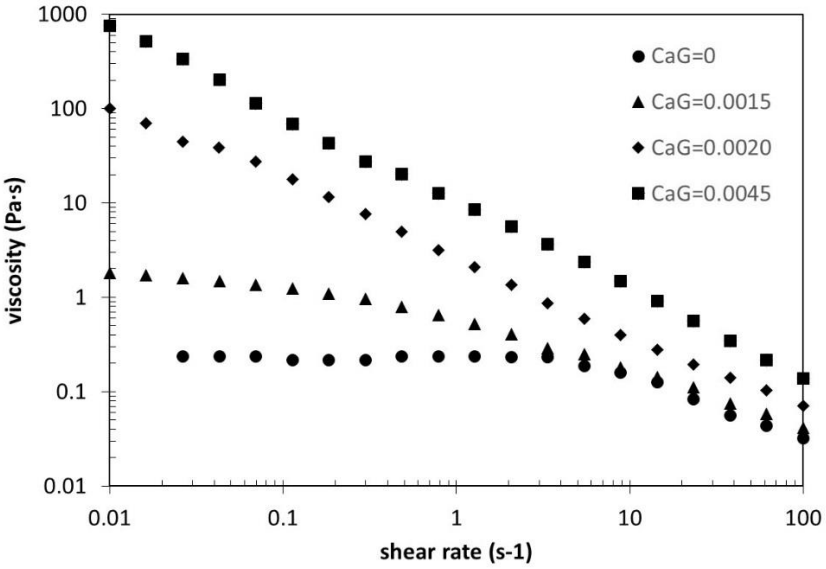

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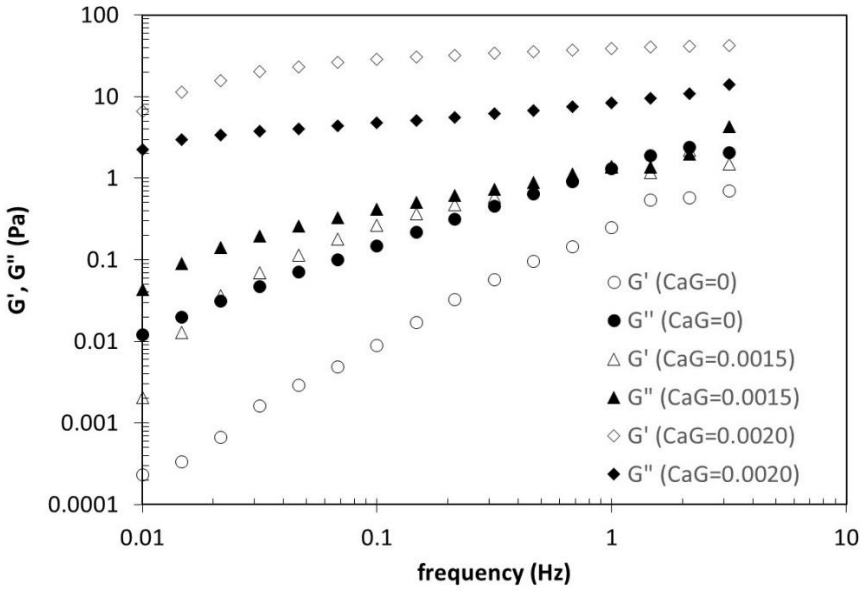

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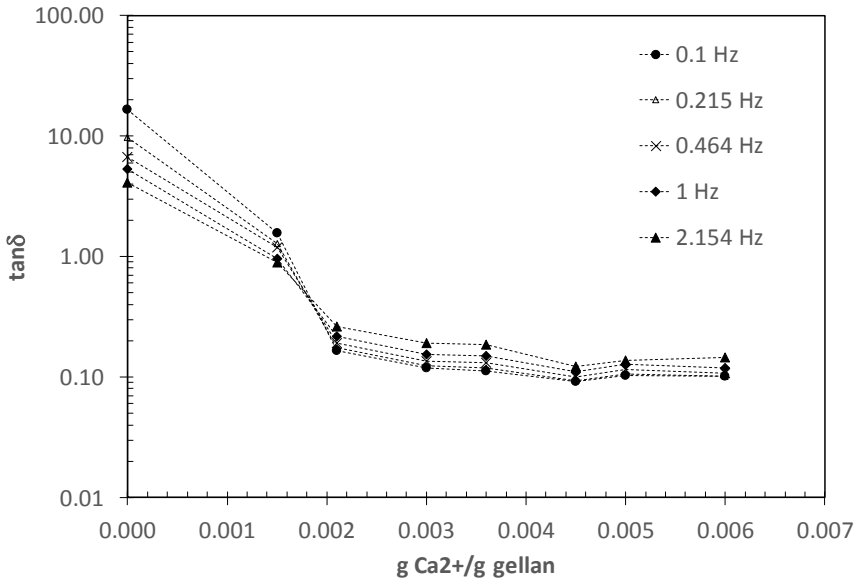

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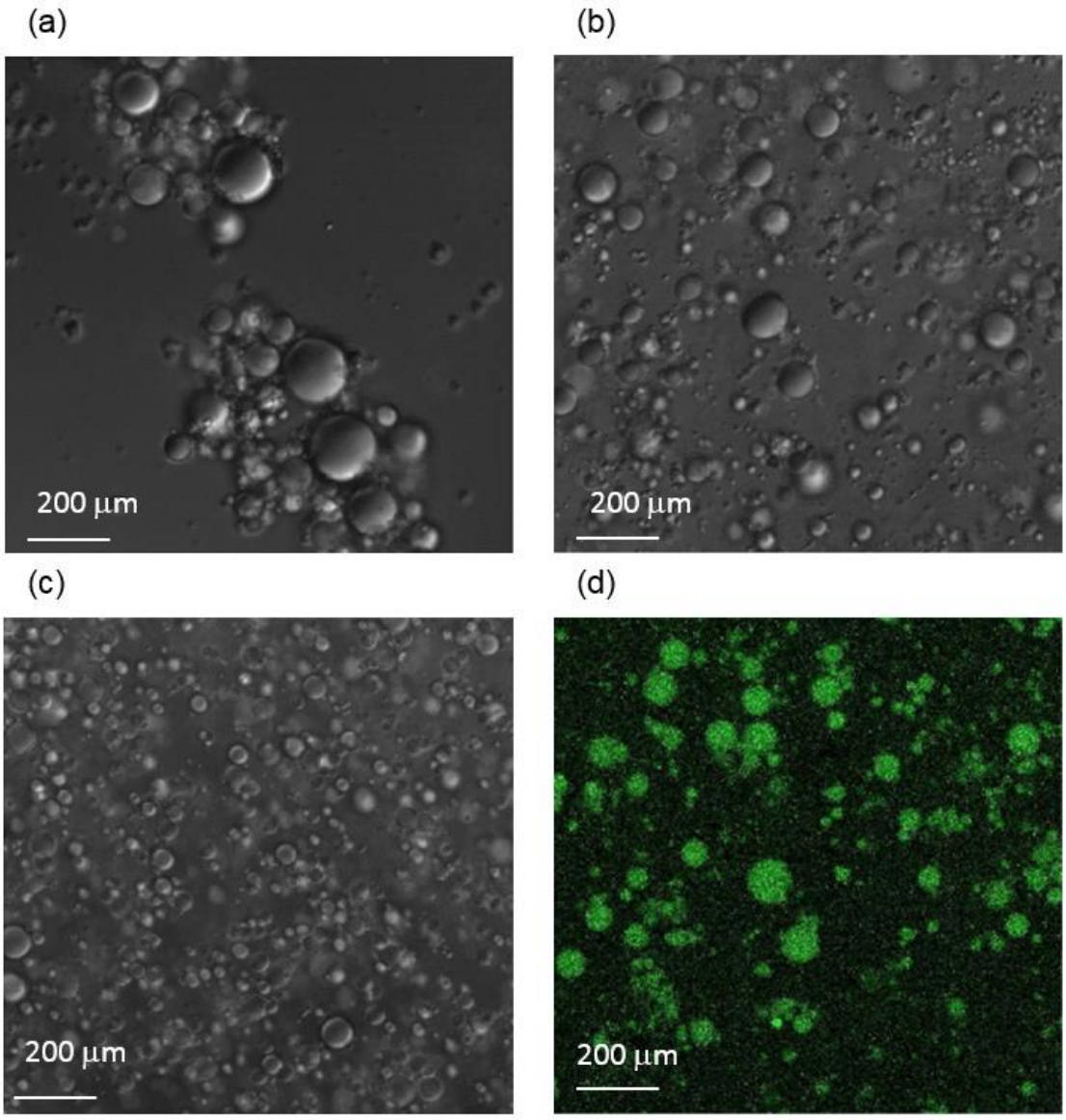

Figure

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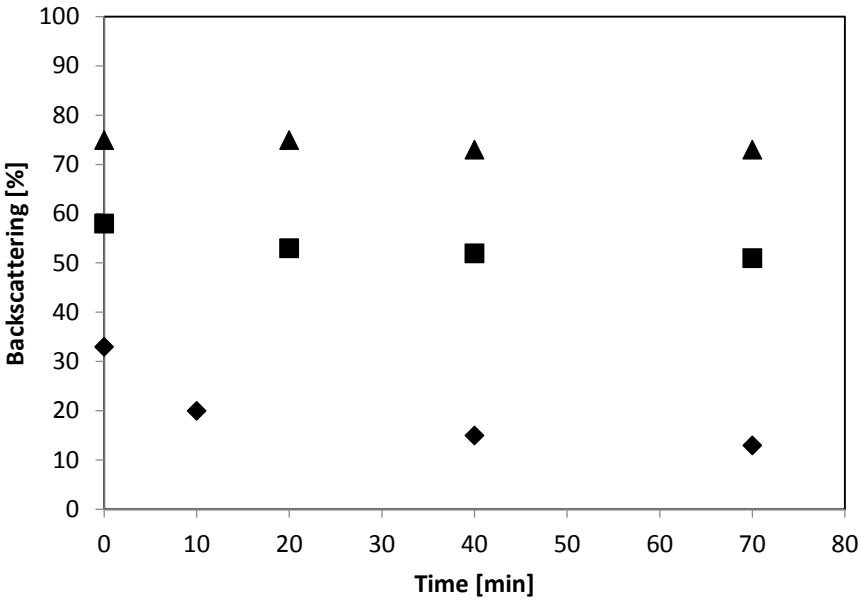

Figure

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