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The effect of carrageenan on the acid-induced aggregation and gelation conditions of quinoa proteins

Natalia Montellano Duran[1], Micaela Galante[1,2], Darío Spelzini[1,2], Valeria Boeris[1,2]

1) Universidad Nacional de Rosario-CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas. Área Fisicoquímica. 2) Universidad Católica Argentina. Facultad Católica de Química e Ingeniería del Rosario

Corresponding author:

Lic. Natalia Montellano Duran

+54 (0) 341 – 4804592/3 /253 | 48004620 | Fax +54 (0) 341-4804598

Suipacha 531 S2002LRK Rosario – SF – Argentina

natalia.montellano@gmail.com

Abstract

This work aimed to study the effect of the presence of Carrageenan (Carr) on the quinoa proteins (QP) structure and acid-induced aggregation. Carr significantly influenced the pH-solubility profile, the effect of the thermal treatment of QP, the fluorescence emission spectra. The QP dispersions were acidified by the addition of glucono-δ-lactone (GDL); the initial soluble aggregates became into smaller structures that close to the isoelectric point, formed larger aggregates due to the neutralization of QP charges. The QP acid-induced aggregation process as well as the size of the aggregates were affected differentially depending on the Carr concentration added. The QP concentration and pH required to form gels were determined by a qualitative procedure absence and presence of different Carr concentrations. The least QP concentration to form gels was decreased by the presence of Carr; in addition, the pH range of gelation was more acid. Acid-induced aggregation process seems to be a competition between QP-QP and QP-Carr interaction, and both biopolymers are synergically responsible for the formation of the gel matrix.

Keywords: quinoa proteins, carrageenan, acid – induced aggregation/gelation

1. Introduction

Functional property of aggregation, protein – protein interactions, is studied for edible proteins in food systems. Proteins have a potential use depending on their physical and physiochemical properties (Totosaus, Montejano, Salazar, & Guerrero, 2002). Vegetable proteins aggregation study is trending topic lately because they can give new characteristics to obtain novel food with different flavor, odor or digestibility. The aggregation stage after denaturation strongly affects the resulting elasticity and mechanical properties of protein-based gels (van Vliet, Martin, & Bos, 2002), so the study of the factors affecting protein aggregation in diluted systems is relevant to assess the effect of these factors on the gel formation and characteristics.

The Andean pseudo cereal, quinoa (*Chenopodium quinoa* Willd.), contains high levels of proteins with an appropriate amino acid balance for human nutrition. Researchers are paying attention to quinoa proteins (QP) since it has a higher protein amount than other grains, high levels of lysine and methionine, and in addition it is gluten-free and non-allergenic (Nongonierma, Le Maux, Dubrulle, Barre & FitzGerald, 2015). Quinoa flour functional and physicochemical properties has been studied lately, but the QP properties are not well known yet (Mäkinen, Zannini, Koehler, & Arendt, 2016).

The structure of QP, mostly globulins, could be influenced by the medium conditions such as pH, ionic strength, and temperature, among others. It is known that the polypeptides from Chenopodin (globulin 11S) subunits, the major protein of quinoa seeds isolate, are soluble at alkaline pH due to their net negative charge (Elsohaimy, Refaay, & Zaytoun, 2015; Steffolani et al., 2015).

Protein functional properties allows solubility, formation of a fine and elastic gel network, or make possible emulsifying activity. These activities can change depending on the medium conditions. Some of QP functional properties has been under research in the last years as emulsifying, foaming and gelation (Elsohaimy et al., 2015; Mäkinen, Zannini, & Arendt, 2015; Ruiz et al., 2016). In gelation property, it was seen that a weak coagulum of QP can be formed depending on the heat-treated pH (Mäkinen, Zannini & Arendt, 2015). The aggregates can be held together not only by hydrogen

bonds and hydrophobic interactions, but also by covalent disulfide bridges, which QP are known to have the ability to form. This behavior explains a stability over a broad pH-range, secondary aggregation leads to a precipitation process at longer time (Mäkinen et al., 2016). Gel characteristics depend on the formation conditions like the thermal treatment (TT) at certain pH to make possible the disulfide bond formation in the aggregation process. The TT of the proteins before acidifying the systems may induce the formation of a stronger network due to covalent interactions, and the gel may have a coarser structure with larger pore size, affecting the textural properties of the gels formed (Nishinari, Fang, Guo, & Phillips, 2014). Disulfide – mediated QP aggregation at different pH levels has been studied previously (Mäkinen et al., 2016). Some researches assured that the denaturation and aggregation mechanisms of quinoa globulins are strongly pH – dependent, while the secondary structure is retained some unfolding occurred at alkaline conditions. Aggregation and gelation processes behavior depends on the conditions due to the QP globulins structural changes (Mäkinen et al., 2015).

One of the acids currently used, and that has replaced bacterial acidification in the dairy industry, is glucono-δ-lactone (GDL), which can acidify the medium by breaking its carboxylic ring in solution. The final pH is a function of the GDL concentration, and the pH decrease depends further on the temperature of the medium (Hidalgo, Riquelme, Alvarez, Wagner, & Risso, 2012).

A commonly used polysaccharide in the food industry is the carrageenan, which is used as a stabilizer, thickener and gelling agent. It comes from a family of sulfated polysaccharides obtained from certain species of red seaweeds. There are three types: kappa (κ-), iota (ι-) and lambda (λ-) carrageenan, they vary on the number of sulfate groups. This work used the ι-Carrageenan (Carr) which carries two sulfate groups per disaccharide over its backbone, with a pKa around 2 (Campo, Kawano, Silva, & Carvalho, 2009).

The aim of this work was to study the effect of the thermal treatment and the presence of Carr on QP structure as well as the acid-induced aggregation process carried out at different conditions. In addition, the qualitative determination of the minimun concentration of thermally treated QP and the pH range required to form acid-

induced gels were determined in the absence and presence of different Carr concentrations.

2. Materials and Methods

2.1. Materials

Partially defatted quinoa flour was purchased from Los Andes (Cochabamba, Bolivia). ι-Carr and GDL were purchased from Sigma Aldrich (Sigma Chemical, St Louis, MO, USA). The rest of the chemical reagents had analytical quality.

2.2. Quinoa proteins

QP recovery was carried out as Abugoch protocol with some modifications (Abugoch, Romero, Tapia, Silva, & Rivera, 2008). Briefly, solubilization was carried out at pH 8.5 and precipitation at pH 4.5. Protein quantification was carried out by Bradford method.

2.3. Solubility

The solubility of QP 10 g/L was assessed as a function of pH by the procedure described by Abugoch (Abugoch et al., 2008) in the absence and presence of Carr. Different concentrations of Carr were assayed (0.00, 0.02, 0.04, 0.06, 0.08, 0.10 and 0.50 g/L) in the pH range (1 – 10) in 10 mM acetate -10 mM phosphate – 10 mM HCl–Tris buffer (Ac-Pi-Tris). Samples were centrifuged at room temperature (1000 g, 10 min) and protein solubility was measured in the supernatant by Bradford method.

2.4. Effect of thermal treatment on QP structure

Thermal treatment of QP in NaOH 0.5 N was carried out at 95°C for 10 minutes in the absence of Carr. The structure of QP thermally treated (QPTT) was characterized by fluorescence emission spectra, determination of hydrodynamic diameter (Dh) distribution and ζ-potential (ζ) by dynamic light scattering (DLS) in the presence and absence of Carr 0.16 g/L. All the dispersions were centrifuged at 10000 g for 10 minutes. Dispersions of soluble QPTT 0.5 g/L were prepared from the supernatant. As a control, QP without TT was also assayed.

The fluorescence emission spectra (300 – 400 nm) were carried out by exciting at 280 nm with an AMINCO-Bowman spectrofluorometer Series 2 (AB2, Spectronic Instruments, Rochester, New York, United States). It was not necessary to correct the spectra for the effect of the internal filter, because a triangular cuvette was used.

The determinations of Dh and ζ were done using a Zetasizer SZ-100 Nanopartica (HORIBA Ltd, Kyoto, Japan). Measurements in the ZetaSizer were the average of 2 complete runs (3 cycles each) performed at 25°C. The ζ was calculated by the ZetaSizer software, it is measured as electrophoretic mobility and converted to ζ-potential measures with the Helmholtz-Smoluchowski equation by the instrument's software (Wall, 2010).

2.5. Acid-Induced Aggregation

2.5.1. Aggregation kinetics process

Acid-induced aggregation of QPTT was studied in dilute regime to avoid gelation. QP (0.5 g/L) samples were prepared at pH 8.5, thermally treated and then added with different Carr concentrations up to 0.5 g/L. Acid-induced aggregation was initiated by GDL addition at different GDL/QPTT ratio (R): 0.17; 0.33; 0.66; 1.00 and 1.33. During the acid-induced aggregation process, turbidity in the visible range (420 to 650 nm) and pH were recorded every 60 seconds. The spectrum was recorded with a diode array Spekol 1200 spectrophotometer (Analytik Jena AG, Jena, Germany). All determinations were made at 30 °C. β value, a parameter related to the particles size in solution, were calculated using the Eq. 1.

$$\beta = 4.2 + \frac{\partial(\log \tau)}{\partial(\log \lambda)} \qquad \text{(Equation 1)}$$

where $\partial(\log \tau)/\partial(\log \lambda)$ is the change of turbidity in function of the wavelength, and 4.2 is the term resultant of equation reductions for the system (Risso, Relling, Armesto, Pires, & Gatti, 2007).

2.5.2. Confocal Laser Scanning Microscopy (CLSM)

Confocal laser scanning microscopy images were obtained with an inverted microscope NIKON C1SiR PLUS (Nikon instruments) using an excitation wavelength of 543 nm and a 560 – 600 nm emission filter. Samples (QPTT 0.5 g/L) were stained by adding an aqueous solution of 0.05 g/L Rodamine B in each sample before acidification with different R. The samples were placed in Lab-tek ® transparent plates to take the images, and incubated at 30°C for 1 h to allow the GDL total hydrolysis.

2.6. Gelation Conditions Determination

Mäkinen et al. (Mäkinen et al., 2015) carried out the gelation of QPTT 20 g/L acidifying with R=0.33. In order to qualitatively determine the minimum QPTT

concentration required to form gels, different concentrations of QPTT were obtained by proper dilution with diluted NaOH at pH 8.5. QPTT concentrations from 3 g/L to 40 g/L were assayed acidifying with a fixed R=0.33. The gel forming conditions were determined by turning the tubes upside down, after 24 h of incubation. When the sample did not fall or slip it was considered gel-formation (Olivos-Lugo, Valdivia-López, & Tecante, 2010).

Once the minimum QPTT concentration required for gelation was determined with a R fixed, it was studied a range for the R required to obtain gels was assessed by setting the QPTT concentration fixed as 24 g/L, twice the minimum concentration of QPTT required for gelation (with an R equal to 0.33, QPTT, 12 g/L) to ensure a wide range where we can change the R to study its effect. The effect of R and Carr concentration (0 to 0.05 g/L) on the required QPTT concentration for gelation was evaluated at fixed QP concentration (24 g/L). Two different conditions were tested:

i) QP was thermally treated and then added with Carr; and

ii) QP was added with Carr, and the mixtures were thermally treated.

The pH was determined after 24 hours of incubation to ensure the complete GDL hydrolysis.

2.7. Statistical analysis

Samples were carried out at least by triplicate and the results were reported as means with standard deviations.

3. Results

3.1. Carr effect on QP solubility

The solubility of QP and QP – Carr dispersions as a function of pH is shown in Fig. 1. In the absence of Carr, QP showed a solubility profile typical for vegetable proteins: highly insoluble around pH 4 to 5 with higher solubility around pH 9 to 11. Similar results have been reported before for soy systems (Ortiz, Puppo, & Wagner, 2004).

QP showed higher solubility in Carr presence, around pH 2.9 and 5.5 (close to the IEP), than QP in solution in the absence of Carr. On the other hand, the presence of Carr produced a decrease in the solubility of QP between pH 1 and 2.9 and between pH 5.5 and 10.

The same behavior was reported when the solubility of soy protein isolate was studied at different pectin concentrations at different pH. Protein solubility increased with pectin addition close to its IEP (between pH 4 and 5) whereas pectin addition decreases the soy protein solubility at higher and lower pH values. Moreover, the presence of pectin also affected the amount and size of the protein aggregates (Jaramillo, Roberts, & Coupland, 2011).

3.2. Effect of Carr and thermal treatment on QP structure

3.2.1. Fluorescence Emmision Spectra Fluorescence emission of samples was determined between 300 and 400 nm, exciting at 280 nm, and the results are shown in Figure 2. The fluorescence emission spectrum of the QP (control sample) has a maximum around 330 nm, which was found previously (Abugoch et al., 2008) in a QP isolate solubilized at pH 9. This indicates that the QP are keeping their native structure. On the other hand, it is observed that the spectrum also shows a second superimposed peak with the first peak at a wavelength of approximately 360 nm. This shoulder could be attributed to the presence of some Trp exposed to the solvent: fluorophores in a polar environment present less energy fluorescence emission.

QPTT showed a redshift of the spectrum when compared to the control, indicating a higher exposure of the fluorescent amino acids to the medium. This would be in agreement with a partial unfolding of the polypeptides because of the thermal treatment effect. In the presence of Carr a redshift of the spectrum occurred, in a smaller magnitude than the observed in samples with the thermal treatment. Carr may induce conformational changes of QP, allowing fluorescent amino acid exposition. It is to be noted that the redshift of the fluorescence emission spectrum induced by the thermal treatment is lower in the presence of Carr than in its absence, suggesting that Carr has a protective effect against the thermal denaturation of the proteins.

3.2.2. ζ – Potential and hydrodynamic diameter

Fig. 3 A shows the ζ-potential measurements obtained for the studied samples. The ζ-potential of the QP sample was approximately – 35 mV. The TT produced a slight decrease to – 42 mV, probably due to the higher exposure of ionizable groups to the solvent, as a result of QP conformational changes. A significant decrease in the ζ-potential was produced in the presence of Carr, probably by the interaction between

QP and Carr or to the decrease of available counterions. TT did not affect significantly the ζ-potential of QP in the presence of Carr, may be due to the main contribution of Carr to the ζ-potential value.

In the D_h measurements (Fig. 3 B) it was seen that the TT increased the size of the QP soluble aggregates, probably due to a decrease in the degree of compactness. The presence of Carr produced an increase in the size of the soluble aggregates, being its effect higher for QP when it is compared with QPTT. The presence of Carr may be inducing the self-aggregation of QP due to the excluded volume effect.

3.3. Acid-induced aggregation

3.3.2. Kinetics of acid-induced aggregation

The effect of R on the acid-induced aggregation process of QPTT (0.5 g/L) was studied. The parameter β, related to the size of the aggregates was measured as a function of the time and pH and the results are shown in Fig. 4.

A similar behavior was observed in the QPTT aggregation process when R was 0.66, 1.00 and 1.33. Firstly, the β value decreased, which could be related to a decrease of the average size of the aggregates, and later, it increased with time and acidification, indicating that they formed larger aggregates at the end of the time assayed. This behavior (decrease and increase of the aggregate size) is characteristic for the acid-induced aggregation of proteins (Hidalgo et al., 2012) and it can be explained considering that the pH variation of the medium induces, in a first instance, a slow dissociation of the soluble protein aggregates. When the pH gets close to the IEP of the proteins, they lose their charges and the repulsion between them decreases, allowing them to interact and form the aggregates, destabilizing the colloids formed in the solution. In the first stage, the aggregates formed could be restructured to form larger aggregates, through hydrophobic interactions (Hidalgo et al., 2012). It is to be noted that the higher the added GDL concentration, the lower the values of the parameter β during the first stage. The minimum β value was reached at pH 6 and, as the R increased, due to the higher acidification rate (higher R values), this minimum was reached faster but always at the same pH. This phenomenon could be explained considering that the increase in the GDL concentration produced the acceleration and magnification of the initial dissociation of the QPTT aggregates. However, different R

values did not alter the pH at which dissociation of initial aggregates occur, suggesting that this phenomenon exclusively depends on the QP charge. Besides, larger final aggregates were produced when R values were higher due to the restructuring phase of the aggregates. This may be due to relatively non-specific protein – protein interactions because there is less time to restructure at faster acidification rate (Mäkinen et al., 2015).

It is to be noted that below pH 6 the QP aggregates increases their size due to protein – protein interaction. In this pH range, the high capacitance of QP promote the QP-QP interaction through charge regulation mechanism (Montellano Duran, Spelzini, Wayllace, Boeris, & Barroso da Silva, 2017).

3.3.3. Fig. 5 shows the evolution of parameter β as a function of pH and time in the presence of different Carr concentrations. It can be observed that Carr had a significant effect on the kinetics of β parameter variation. The presence of Carr delayed the dissociation of the aggregates, not only the time at which the smallest aggregates (β minimum value) was obtained, but also the pH in which this phenomenon was observed. When the lower Carr concentrations tested (0.02 and 0.04 g/L), the β vs time and pH profiles were similar to the QP in the absence of Carr. On the other hand, at Carr intermediate concentrations (0.06 and 0.08 g/L), the pH at which the smaller aggregates are formed were significantly decreased and the subsequent aggregation step did not occur. The higher Carr concentrations (0.1 and 0.5 g/L) produced a different behavior, being the stage of dissociation of the aggregates not observed in most cases. Confocal laser scanning microscopy (CLSM)

The confocal images of QPTT and QPTT – Carr obtained by CLSM after acidifying with different R values are shown in Fig. 6. It was found that the morphology of the aggregates depends on the added amount of GDL. The initial pH of the systems was 8.5 and a higher R reach lower pH final values. The aggregates formed in the systems with higher R values have a particulated form, typically seen in aggregates formed at acidic pH near to the IEP. There are two positions about how the aggregates form: 1) the particles are formed by micro phase separation of smaller aggregates (Ako et al., 2009) and 2) a nucleation and growth model explain the formation of the particles

(Bromley, Krebs, & Donald, 2006). Both models explain different paths for the formation of bigger particles from smaller ones in solution. At higher QPTT concentrations both paths can lead to the gel matrix formation by protein – protein interactions. Comparing the results for the same R value, aggregates observed in QPTT and QPTT – Carr systems have similar form, but the aggregates in the QPTT – Carr systems are less interconnected than those in absence of Carr.

3.4. Acid-induced gelation

The acidification at higher QPTT concentrations is expected to result in a gel network formation, if conditions are appropriate. The gel properties depend on the protein concentration, the rate of pH decrease, the final pH of the system and the presence of cosolutes or copolymers. The minimum QPTT concentration required to form a gel was determined to be 12 g/L with R: 0.33. Above the critical gel concentration a solid system is formed that is sustainable, but at lower QPTT concentrations, the system flows or collapses under gravity. Considering this result, the pH and acidification conditions required to form gels from a suspension of QPTT 24 g/L were studied. Close to the IEP, up to the critical protein concentration, the acid-induced aggregation process leads to gelation or precipitation, the gels or precipitates consist of agglomerates of large spherical particles (Zhang & Vardhanabhuti, 2014). Fig. 7 A shows the pH values measured for each R values tested and it distinguishes between the ones that formed gels (filled) and those which not (empty). As expected, an inverse relationship was observed between the final pH obtained and the GDL concentration added in the systems. It was observed that the systems were able to form a gel if the pH was above 2.9 and below 5.5, which is achieved using an R between 0.26 and 1.3. As was previously discussed, this pH range is in agreement with that determined for the charge regulation mechanism (Montellano Duran et al., 2017). If the pH is extremely acid or the rate of acidification is high, the proteins precipitate, being unable to form soluble aggregates or gel structures. On the other hand, as was discussed before, if the medium is not acid enough, proteins do not reach the IEP and their aggregation do not take place.

In addition, the minimum QPTT concentration required to form gels as a function of R were evaluated and the results are shown in Fig. 7 B. It is to be noted that higher

QPTT concentrations were required for the gel formation when R increased. This could be explained since more protein would be necessary to neutralize the protons released by the GDL to reach an appropriate pH to form the gel matrix.

Different characteristics can be obtained adding a polysaccharide to the protein system which leads to the gel formation. Depending on the protein – polysaccharide interaction and the degree of thermodynamic compatibility of the mixed systems, gels with particular structures may be obtained (Picone & da Cunha, 2010). This affects not only the gels rheology, microstructure, appearance and water holding capacity but also the minimum conditions required for gelling. The pH range (or added GDL, R) required for gels formation from [QPTT] = 24 g/L with different Carr concentrations was tested, as shown in Fig. 8. Two different thermal treatments were assayed as section 2.6 propose. There was not much difference between the systems heated together with the ones heated separately. The presence of Carr modified the maximum pH at which gels were formed: a more acid pH was required for gelation. This is in agreement with the aggregation experiments: the presence of Carr decreased the pH at which the rate of aggregation exceeds the rate of dissociation of the aggregates, QPTT systems added with Carr required a more acidic medium to form aggregates, and subsequently, gels. This behavior was noticed for all Carr concentrations tested, except for the higher one (0.5 g/L). This could be because the presence of a higher Carr concentration modified the QPTT aggregates stability and this changed the observed behavior. The minimum QPTT concentration required to form gels was determined in the presence of different Carr concentrations, using R: 1, as shown in Fig. 9. The minimum QPTT concentration to form gels decrease in the presence of Carr. An increase in the polymer concentration favors the formation of aggregates due not only to higher total amount of polymer in the systems but also to their interaction (Picone & da Cunha, 2010).

4. Discussion

The effect of Carr on QP solubility may be explained considering the interaction between them. This interaction was previously studied and it was found that below the IEP of QP, both biopolymers in the system carries opposite electric charge, allowing them to interact through coulombic attraction whereas around the IEP the presence of Carr modulated the charge of QP allowing their electrostatic interaction. At neutral and

alkaline pH the presence of Carr diminishes the QP solubility due to the excluded volume effect (Montellano Duran et al., 2017; Steffolani et al., 2015).

The QP thermal treatment increased the protein aggregates size due to protein denaturalization and the presence of Carr decreased the ζ-potential due to the Carr charges.

Acid-induced aggregation and gelation process begins at pH 8.5, where QP solubility in the presence of Carr decrease. QP solubility increase in the presence of Carr when the system get close to the IEP (between 2.9 and 5.5) during the acidification. This pH range is also where QP and Carr interacts electrostatically by the charge regulation mechanism (Montellano Duran et al., 2017).

Analyzing the β parameters during the QPTT acidification, in absence of Carr, it is remarkable that the R value may have an important effect on the structure and characteristics of the QPTT gels. In fact, for the lowest R assayed (0.17), there were no significant changes in β values during evolution, indicating that the dissociation and subsequent aggregation processes did not occur. This behavior may be due to the protons quantity, leading to an R insufficient to neutralize the QP charges, in the time assayed. Thus, the medium pH did not reach pH 6, so the initial dissociation could not take place and neither could the further aggregation. When R: 0.33 was assayed, the QP aggregate size decreased close to pH 6 but the restructuring phase was not observed since the pH reached is not close enough to the IEP. In the presence of Carr, a decrease in the pH required to dissociate the initial aggregates was produced because of an increase of the negative charge. In addition, the presence of Carr avoided the formation of larger aggregates once the initial aggregates were dissociated; it is probably that the aggregation process (QP – QP interaction) was competing with the complexation process (QP – Carr interaction). This is in agreement with the fact that as higher the Carr concentration was, the β value obtained was lower.

Two stages may be distinguished during the acid-induced aggregation of QP: 1) in alkaline, neutral an slightly acid media (from pH 8.5 to pH 6.0), the partial neutralization of QP takes place and the size of the soluble aggregates decrease, 2) the pH gets close to the IEP of QP, the charge regulation mechanism allows the QP – QP interaction and the size of the soluble aggregates increases. The presence of Carr

change this behavior, leading the aggregation at lower pH or not going through the decrease in size of the initial aggregates.

The critical QPTT concentration to form a gel network was found to be 12 g/L at R: 0.33 whereas an R between 0.27 and 1.3 was necessary to obtain a gel with a QPTT concentration of 24 g/L. The final pH of these gels were between 2.9 and 5.5, denoting that the QP charges are in the pH range where the QP have the capacity to regulate their charges to interact between them.The presence of Carr does not compensate the QPTT concentration decrease required to form gels, even if the total polymer concentration (QP + Carr) in the mixture is considered. This could be because there is an interaction between QPTT and Carr that promotes the acid-induced gelation.

5. Conclusions

QP solubility is diminished in the presence of Carr in the pH range between 1 and 2.9 and 5.5 and 10, and it is increased between 5.5 and 10, where QP have the charge regulation mechanism. The presence of Carr induces conformational changes in QP increasing the size of the aggregates and produced the increase in the magnitude of the negative ζ – potential. In addition, Carr protects the QP from the TT when it is present in the system.

Both in the acid-induced aggregation and gelation process the presence of Carr makes necessary a more acid pH to form the larger aggregates and gel networks. The presence of Carr decrease the QPTT concentration needed to form the network.

During the acid-induced aggregation process seems to be a competition between the QP – QP interaction and the QP – Carr interaction; however, at higher concentration of QP, both biopolymers are synergically responsible of forming the gel matrix.

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Figure captions

Figure 1: *QP solubility at different Carr concentrations in the pH range between 1 and 10. Temperature 30°C. Buffer Ac-Pi-Tris (10 mM for each).*

Figure 2: *Fluorescence emission spectra (300 – 400 nm) of QP and QP – Carr with and without TT at pH 8.5. Buffer Ac-Pi-Tris 10 mM. Temperature 30°C. λ_{exc} = 280 nm.*

Figure 3: *A) ζ – potential, and B) Hydrodynamic diameter of QP and QPTT samples in the absence and presence of Carr. Temperature 25°C. Buffer Ac-Pi-Tris 10 mM, pH 6.*

Figure 4: β parameter evolution as a function of pH and time in a solution with different R values. R=0.17 (-●-), R=0.33 (-▼-), R=0.66 (-■-), R=1.00 (-◆-), R=1.33 (-▲-). QPTT concentration 0.5 g/L. Temperature 30°C. A) β parameter in function of the time (minutes) and pH, B) β parameter in function of the time (minutes), C) β parameter in function of the pH.

Figure 5: β parameter evolution during the acid-induced aggregation of the QPTT (0.5 g/L), at different R values as a function of pH in a solution with different Carr concentrations: [Carr]=0 g/L (-●-), [Carr]=0.02 g/L (-▼-), [Carr]=0.04 g/L (-■-), [Carr]=0.06 g/L (-◆-), [Carr]=0.08 g/L (-▲-), [Carr]=0.1 g/L (-★-), [Carr]=0.5 g/L (-⬟-). Temperature 30°C. A) R=0.33, B) R=0.66, C) R=1.00, D) R=1.33.

Figure 6: *Confocal laser microscopy images from QPTT (0.05 g/L) and QPTT (0.05 g/L) + Carr (0.04 g/L) with different R.*

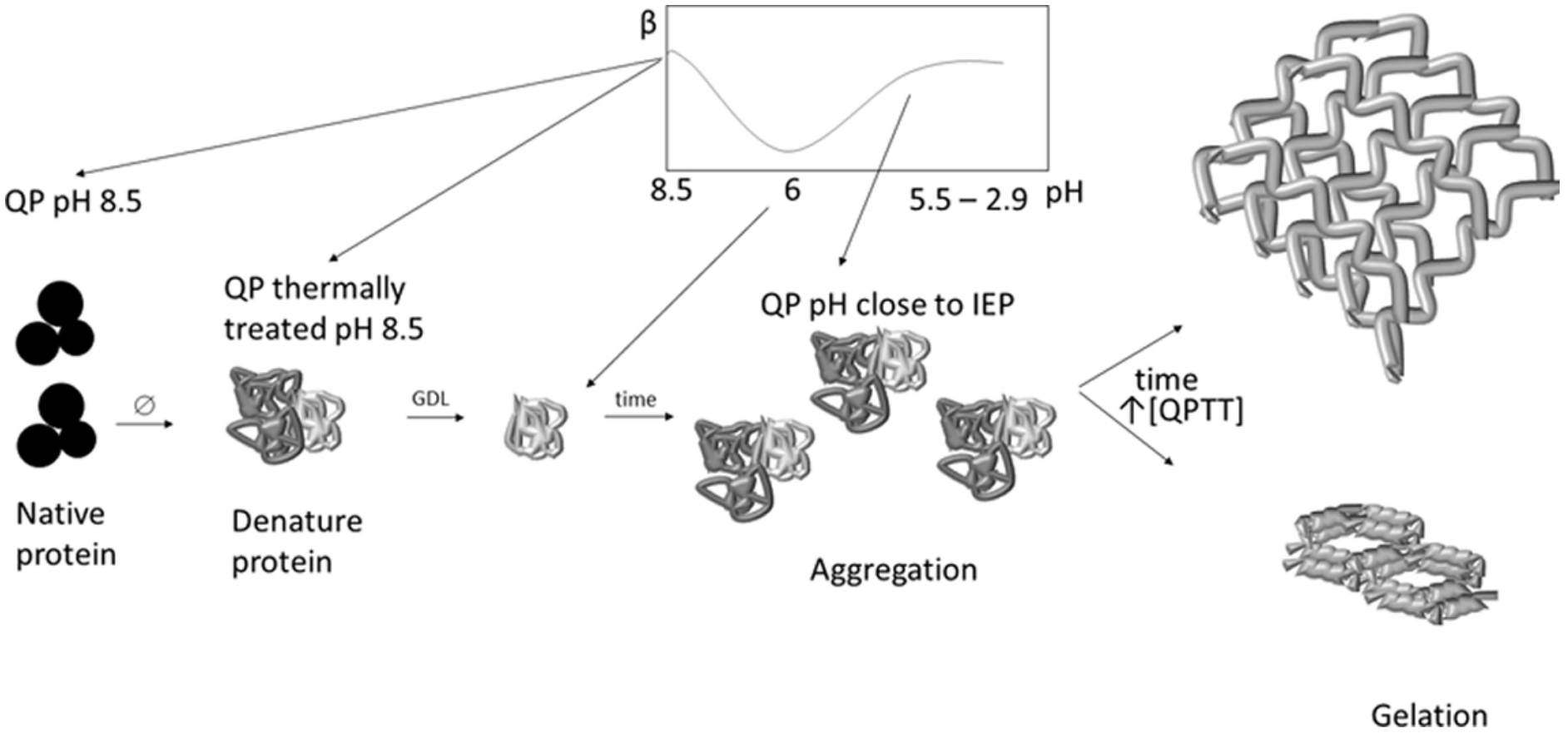
Figure 7: *A) Gel formation capacity and final pH reached for QPTT concentration 24 g/L at different R. B) QPTT concentration needed to form gels at different R values.*

Figure 8: *Minimum and maximum A) R necessary to form gels with the different Carr concentrations. B) pH at which gels are formed with the different concentrations of Carr. Temperature 30°C.*

Figure 9: *QPTT minimum concentration necessary to form acid-induced gels as a function of Carr concentration. Initial pH 8.5. Temperature 30°C. R: 1.*

Highlights

1) Quinoa proteins (QP) aggregate between pH 2.9 and 5.5
2) Higher acidification rate increases the size of the QP aggregates
3) The acid needed to form a gel network depends on the QP concentration used
4) In the presence of carrageenan, a more acid pH is required to aggregate QP



Graphics Abstract

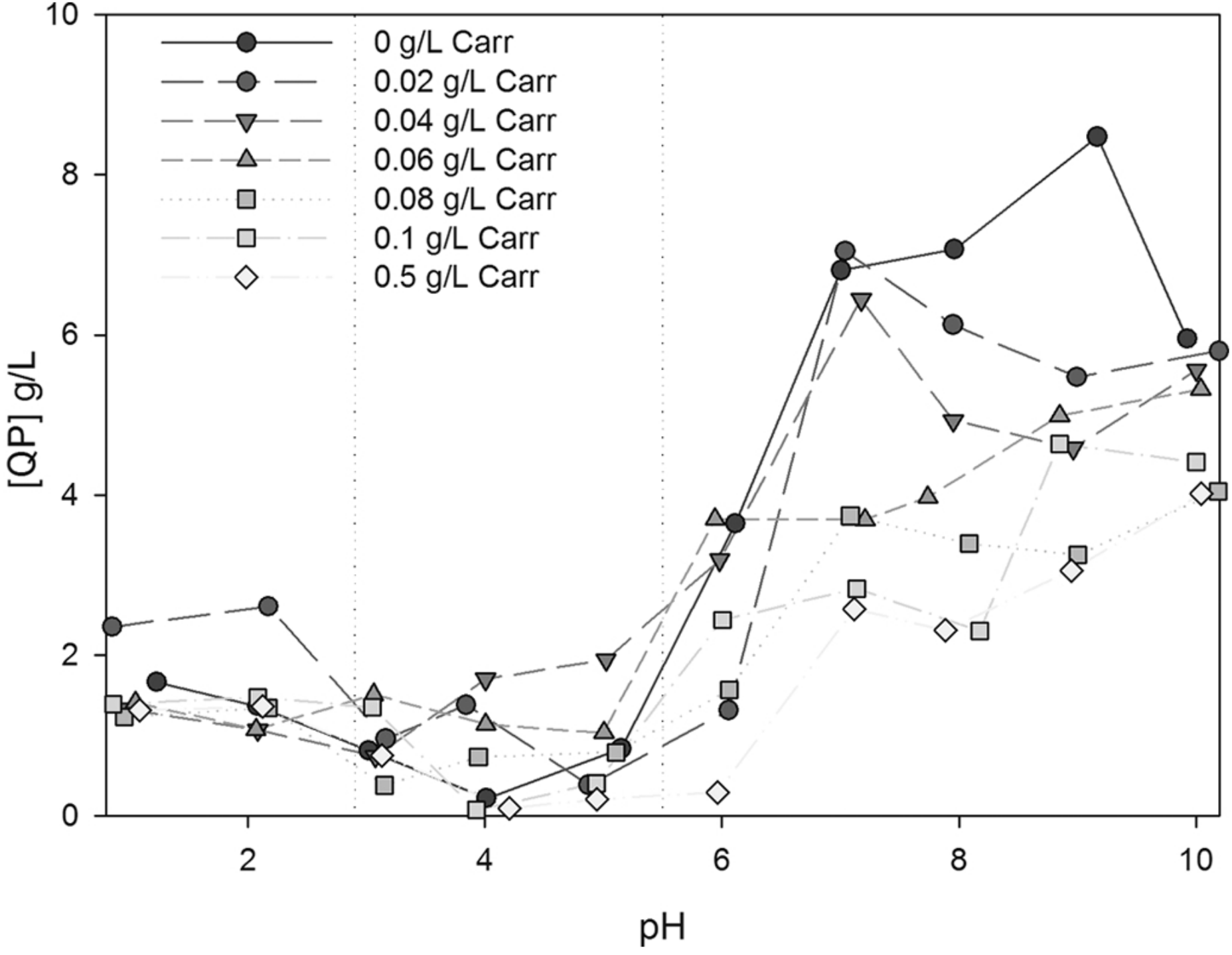

0 g/L Carr
0.02 g/L Carr
0.04 g/L Carr
0.06 g/L Carr
0.08 g/L Carr
0.1 g/L Carr
0.5 g/L Carr
[QP] g/L
pH
0
2
4
6
8
10

Figure 1

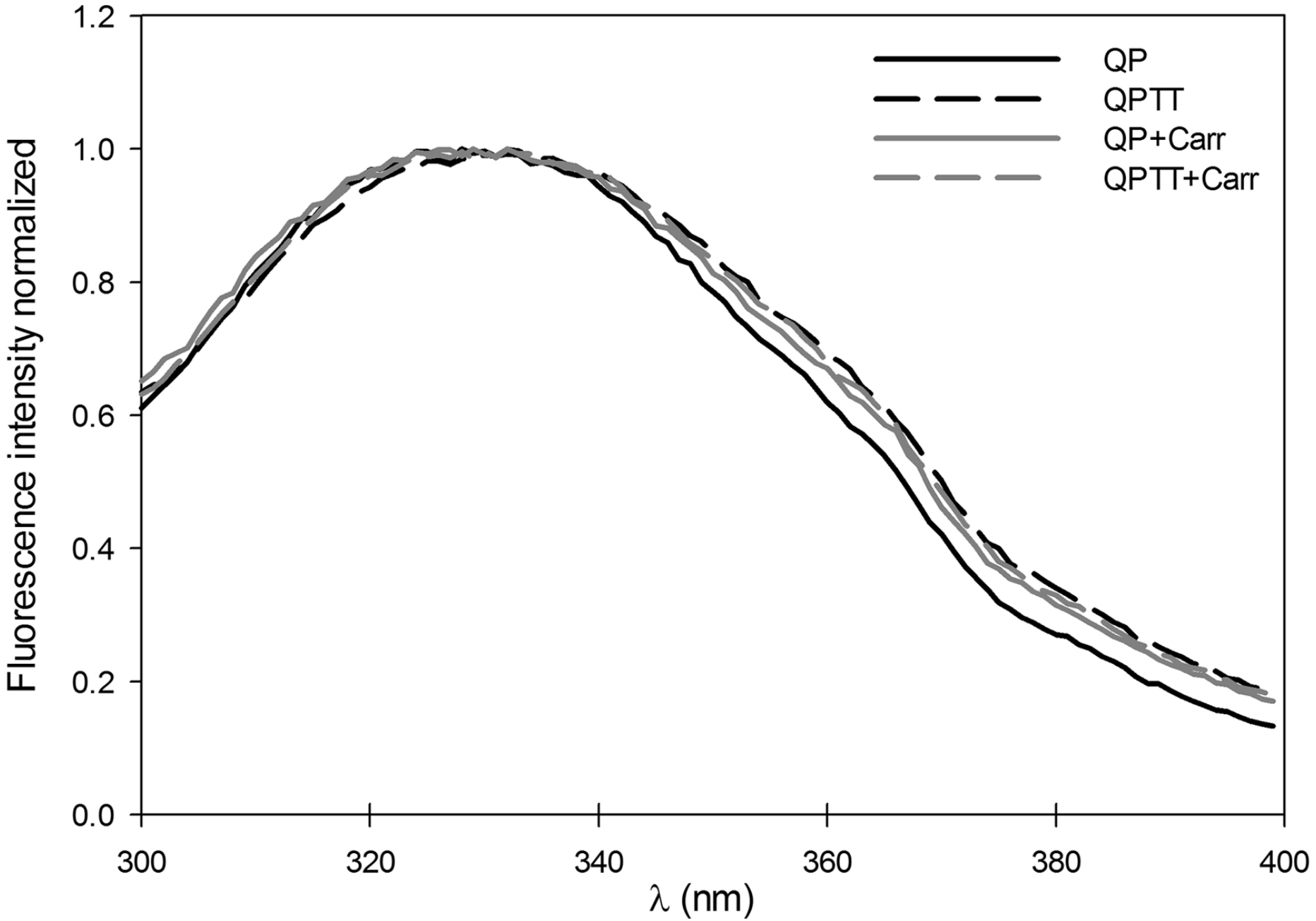
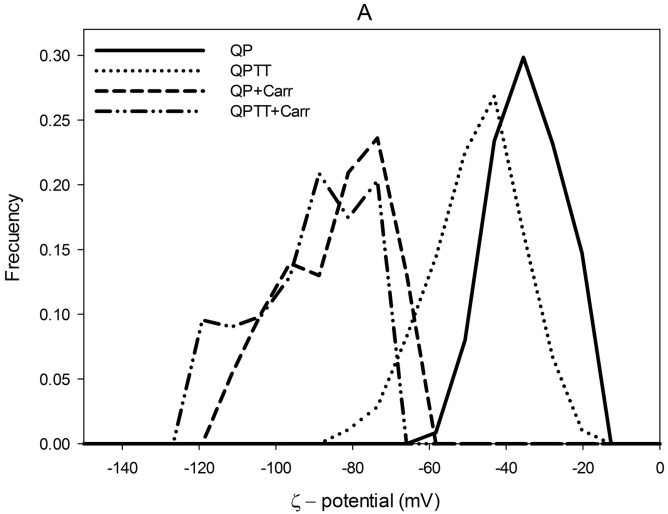

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Fluorescence intensity normalized
300
320
340
360
380
400
λ (nm)
QP
QPTT
QP+Carr
QPTT+Carr

Figure 2


A
QP
QPTT
QP+Carr
QPTT+Carr
Frecuency
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-140
-120
-100
-80
-60
-40
-20
0
ζ – potential (mV)

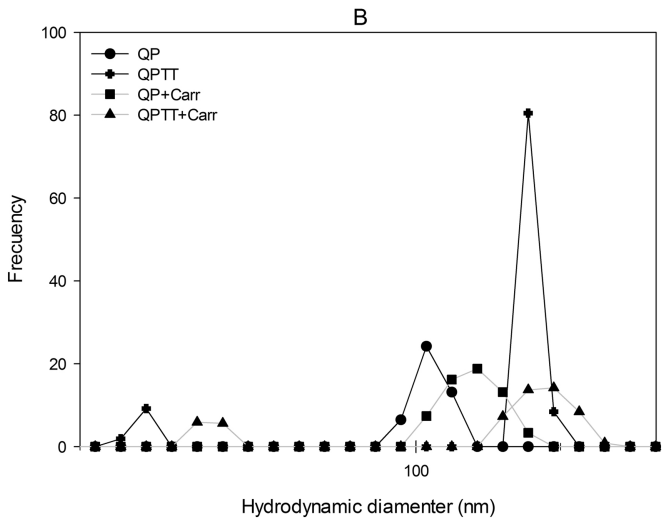

B
QP
QPTT
QP+Carr
QPTT+Carr
Frecuency
100
80
60
40
20
0
100
Hydrodynamic diamenter (nm)

Figure 3

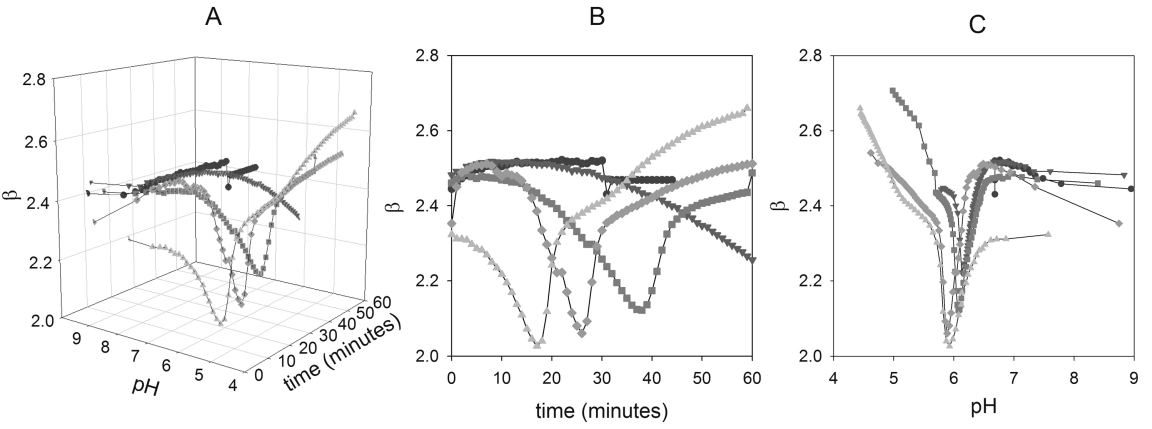


Figure 4

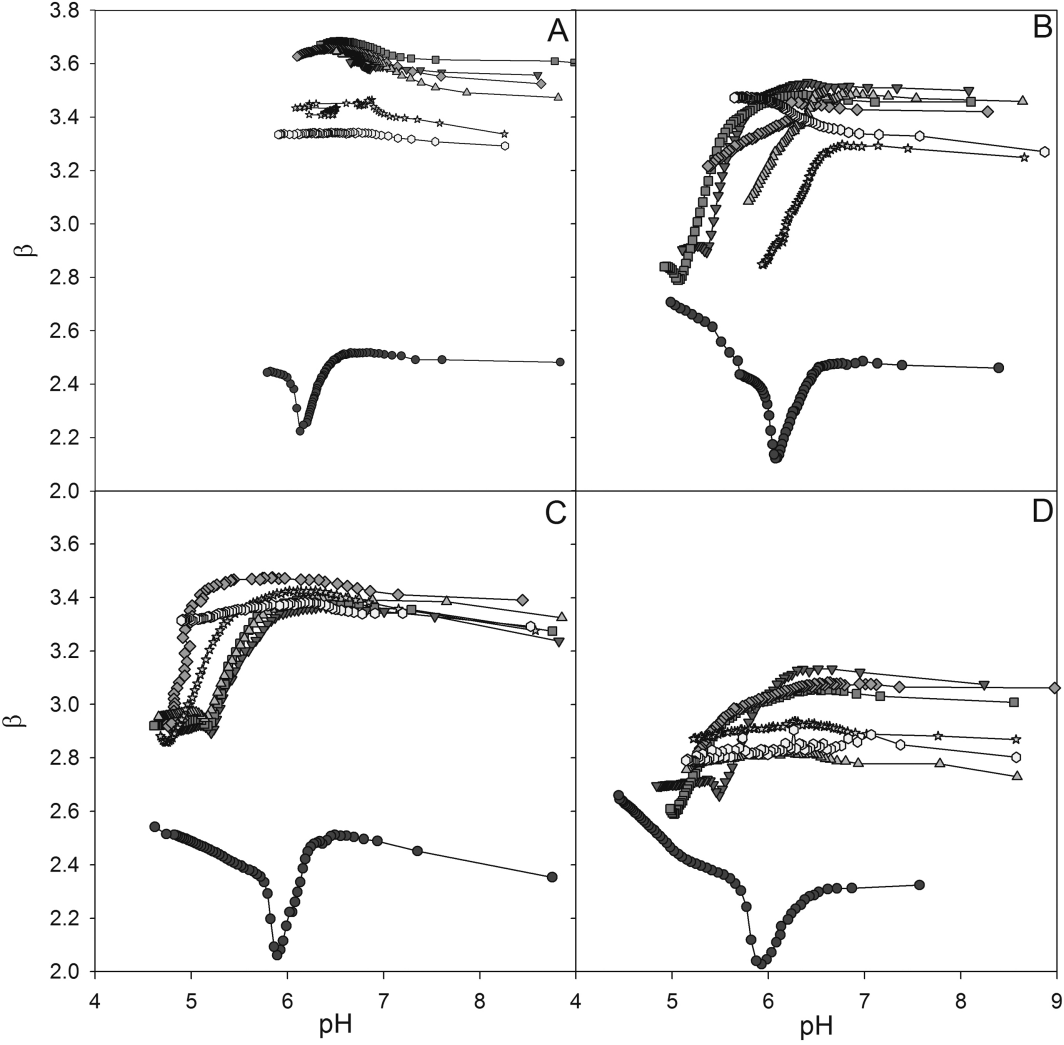


Figure 5

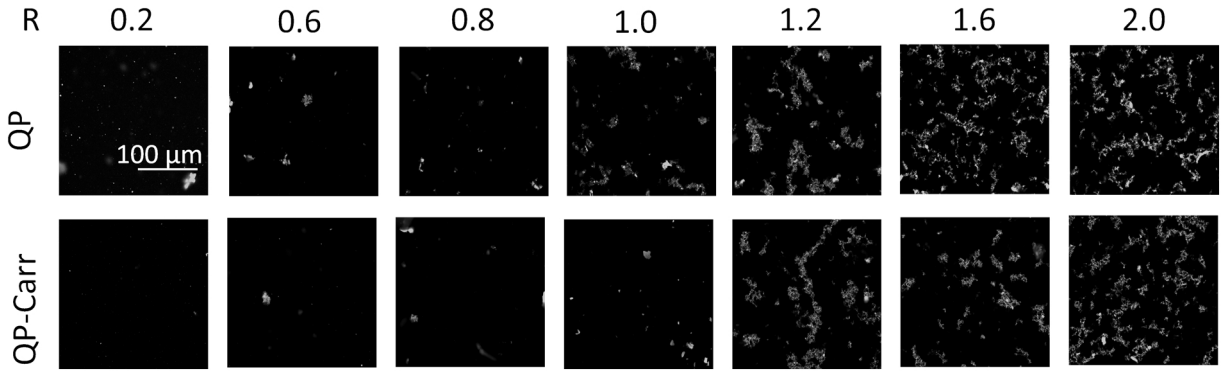


Figure 6

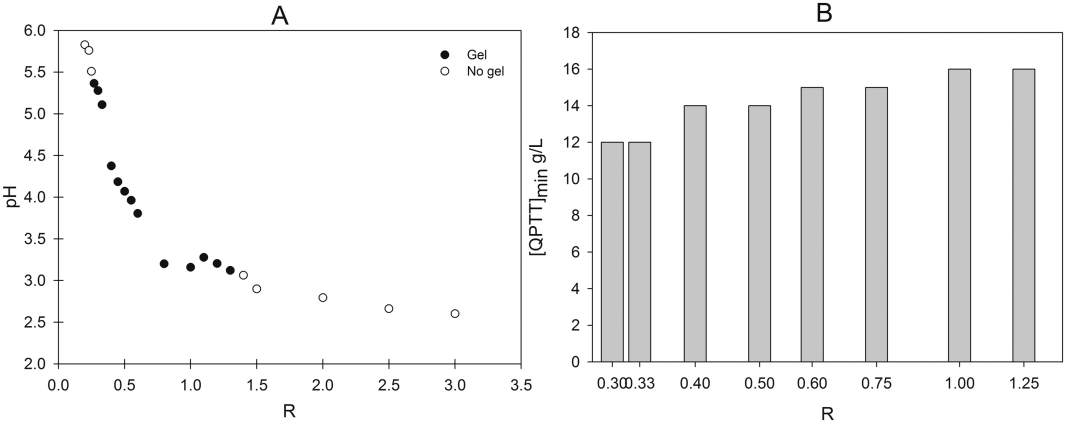

A
pH
R
Gel
No gel
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
B
[QPTT]min g/L
R
18
16
14
12
10
8
6
4
2
0
0.30
0.33
0.40
0.50
0.60
0.75
1.00
1.25

Figure 7

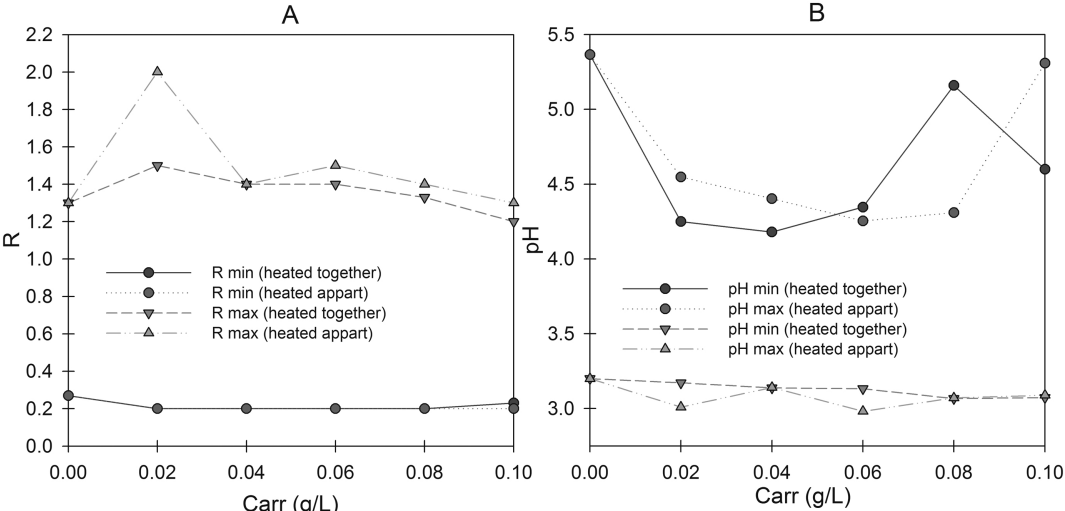

A
R
Carr (g/L)
R min (heated together)
R min (heated appart)
R max (heated together)
R max (heated appart)
B
pH
Carr (g/L)
pH min (heated together)
pH max (heated appart)
pH min (heated together)
pH max (heated appart)

Figure 8

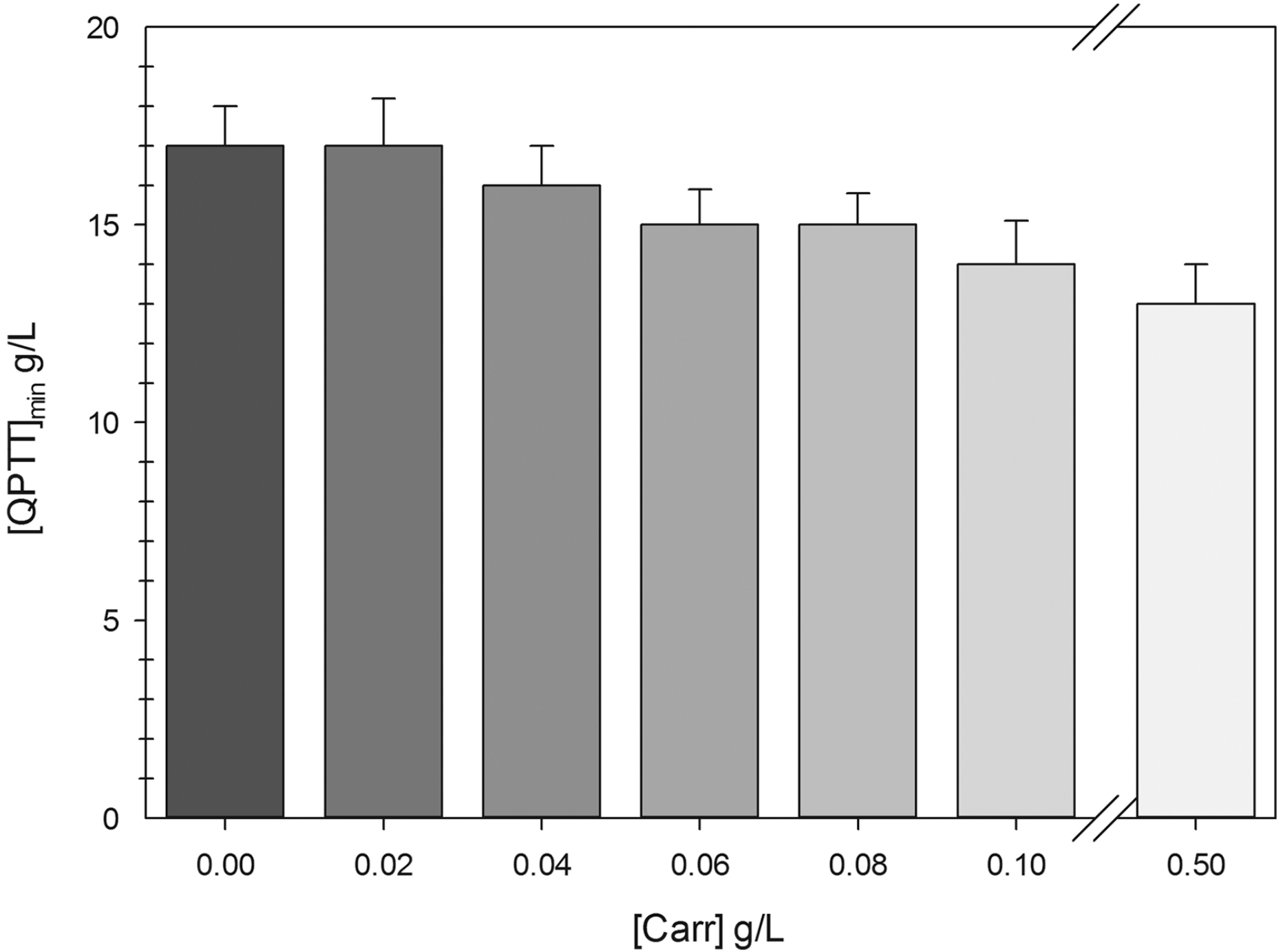


Figure 9