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Chitosan–gellan electrostatic complexes: Influence of preparation conditions and surfactant presence

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ABSTRACT

Nanoparticles were obtained by electrostatic complexation between chitosan and gellan gum at different polysaccharide ratios. The effect of the chitosan:gellan ratio on the particle charge and particle size distribution was determined by dynamic light scattering measurements. The particle stability was studied during storage in an aqueous medium at 25 °C for 100 h. The effect of the preparation procedure (mixing steps) on the characteristics of the complexes was also determined. In addition, the influence of a nonionic surfactant (polysorbate-20) on the chitosan:gellan electrostatic complexes (PECs) was evaluated. The charge of the PECs depended on the polysaccharide ratio. During storage, structural reorganization of the complexes was observed. The mixture protocol was a determinant factor for PEC size. Multilayered particles formed by a 2-step mixing of polysaccharide solutions showed a considerable increase in size as compared to the complexes formed by a 1-step mixing. The PEC size, count rate and zeta potential were not affected by the presence of polysorbate-20.

1. Introduction

Many techniques have been used for the production of nanoparticles, such as solvent evaporation (Desgouilles et al., 2003), interfacial polymerization (Ibrahim, Bindschaedler, Doelker, Buri, & Gurny, 1992) and emulsification methods (Battaglia et al., 2007; Lv, Zheng, & Tung, 2005; Qi, Chen, Huang, Jin, & Wang, 2012). However, most of these approaches involve the use of organic solvents, high temperatures and elevated shear rate, which limit their application in the encapsulation of labile compounds. Thus the electrostatic complexation shows relevant advantages, since it is a biocompatible process, requires low energy and does not use cross-linking agents (Jintapattanakit et al., 2007; Lankalapalli & Kolapalli, 2009). The electrostatic complexes (PECs) are formed by the association of oppositely charged polyelectrolytes (Amaike, Senoo, & Yamamoto, 1998) and lead to particles with dimensions on a colloidal level, generating optically homogeneous and stable nano-dispersions (Mao, Bakowsky, Jintapattanakit, & Kissel, 2006; Sun, Mao, Mei, & Kissel, 2008).

The most commom food PEC is formed from sodium alginate and chitosan. Chitosan is a nontoxic, bioadhesive and biocompatible cationic polysaccharide obtained by the partial deacetylation of chitin. Its linear structure is composed of three reactive functional groups, an amino group and primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively (Claesson & Ninham, 1992; Shahidi, Arachchi, & Jeon, 1999). When positivelly charged, the amino groups are promising sites for electrostatic interactions (Gomez, Ramirez, Neira-Carrillo, & Villalonga, 2006). The molecular weight of chitosan varies from 1 to 1000 kDa depending on its degree of acetylation (DA) (George & Abraham, 2006) and is 10 times higher than that of globular proteins (Payne & Raghavan, 2007). This polysaccharide is widely used to form PECs due to its abundance and mucoadhesive properties which, enhance absorption of the bioactive in the intestine. However, nanoparticles composed of chitosan alone are unstable to low pH values and may release the bioactive earlier than expected due to its fast dissolution in the stomach (Hamman, 2010). Such instability can be overcome by complexing the chitosan with sodium alginate or another anionic polysaccharide resistant to low pH values, such as gellan gum (Norton, Cox, & Spyropoulos, 2011; Picone & Cunha, 2011).

Gellan gum is a nontoxic, biocompatible and biodegradable heteropolysaccharide produced by *Pseudomonas elodea* (Jansson, Lindberg, & Sandford, 1983; Murano, 1998; Prajapati, Jani, Moradiya, & Randeria, 2013; Wen, Yang, Hu, Chen, & Jia, 2008) which has received increasing attention due to its capacity to form strong gels, even at concentrations as low as 0.2% (w/v) (Moritaka, Nishinari, Taki, & Fukuba, 1995; Picone & Cunha, 2011; Yamamoto & Cunha, 2007). Its structure presents a wormlike shape (Dentini, Coviello, Burchard, & Crescenzi, 1988) and consists of tetrasaccharide repeating units of D-glucose, D-glucuronic acid, D-glucose and L-rhamnose (Jansson et al., 1983). Gellan shows a coiled conformation at high temperatures and aggregates into double helices as the temperature decreases (Picone & Cunha, 2011). The

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helical conformation shows substantial stiffness (Dentini et al., 1988) and its persistence length is reported as 98 nm at 25 °C (Takahashi et al., 2004). The main factor that distinguishes gellan gum from other polysaccharides is its gel forming capacity at low pH values. It makes chitosan–gellan complexes a promising enteric delivery system: the gellan gum protecting the bioactive from the low pH of the stomach whilst the chitosan would promote mucoadhesion of the complex to the intestinal tissues and the controlled release of the bioactive component.

Several variables affect the formation mechanisms and stability of the polyelectrolyte complexes in water, such as the ionic strength, pH, polyelectrolyte concentrations and their characteristics such as molecular weight, charge density, flexibility and the presence of hydrophilic or hydrophobic moieties in the polymers (Vidal, Fagundes, Menezes, Ruiz & Garcia, 2005). Surfactants are amphiphilic molecules that readily absorb at surfaces, thereby decreasing the interfacial tension. Despite intensive research aimed at understanding surfactant–polymer interactions (Grant, Cho, & Allen, 2006; Grant, Lee, Liu, & Allen, 2008; Groot, 2000; Naderi, Claesson, Bergström, & Dėdinaitė, 2005; Pepić, Filipović-Grčić, & Jalšenjak, 2008; Pepić et al., 2009; Ziani, Henrist, Jerome, Aqil, Mate, & Cloots, 2011), their influence in the complexation mechanisms of biopolymer systems is still not clear.

The aim of this work was to evaluate the complexation between chitosan and gellan gum and to investigate the characteristics of the PECs obtained. The stability of the complexes was evaluated with time. The effects of the preparation conditions and of the presence of a nonionic surfactant on the final characteristics of the PECs were also studied.

2. Materials and methods

Deacylated gellan gum powder (Kelcogel® F) (250 kDa, 3.42%, w/w, moisture content) was kindly donated by Kelco Biopolymers (San Diego, CA). Low molecular weight chitosan (150 kDa, 75–85% deacetylated) was purchased from Sigma–Aldrich Co. (St Louis, EUA) and polyoxyethylene sorbitan monolaurate, also known as polysorbate-20 or Tween-20, was obtained from Sinth (Brazil).

2.1. Preparation of stock solutions

The chitosan (C) stock solution (1%, w/v) was prepared by the dissolution of chitosan powder in 100 mM sodium acetate buffer (pH 3) and stirred overnight at 25 °C. The 0.7% (w/w) gellan gum (G) stock solution (pH 5.3) was prepared by stirring the gellan powder in deionized water at 80 °C for 30 min in a jacketed vessel, followed by cooling to 10 °C in an ice bath. Each stock solution was diluted in deionized water to a final concentration of 0.01% (w/v).

2.2. Sample preparation

The 0.01% (w/v) polysaccharide solutions were mixed by magnetic stirring at the final concentrations presented in Table 1, and the pH adjusted to 4.5 by the addition of 0.1 M HCl. After adjustment of the pH, samples were stirred for 2 h at 25 °C before analysis.

The protocol of particle formation was tested by preparing multilayered systems (M1C:9G and M2C:8G). The pre-mixtures were first prepared by the conventional method mentioned above. After 2 h, extra volumes of 0.01% (w/v) chitosan or gellan solutions were added to the pre-mixtures to change their concentration ratio from 1C:9G to M2C:8G and from 2C:8G to M1C:9G, respectively. The pH of the samples was reajusted and the samples stirred for a further 2 h at 25 °C before analysis.

The effect of the surfactant concentration on the chitosan–gellan interactions was studied using the 3C:7G and 2C:8G systems. The samples were prepared by the conventional method at the surfactant concentrations presented in Table 1, as detailed before.

Table 1
Composition of systems.

System	Chitosan (%w/v) × 10^3	Gellan (%w/v) × 10^3	Surfactant (%w/v) × 10^3
0C:1G	0.0	10.0	
1C:9G	1.0	9.0	
2:C8G	2.0	8.0	
3C:7G	3.0	7.0	0.0
4C:6G	4.0	6.0	
5C:5G	5.0	5.0	
1C:0G	10.0	0.0	
3C:7G:0.05S			0.05
3C:7G:0.1S			0.1
3C:7G:0.2S	3.0	7.0	0.2
3C:7G:0.5S			0.5
3C:7G:1S			1.0
2C:8G:0.05S			0.05
2C:8G:0.1S			0.1
2C:8G:0.2S	2.0	8.0	0.2
2C:8G:0.5S			0.5
2C:8G:1S			1.0

2.3. Particle size, zeta potential and quantity of particles

The size distribution and zeta potential of the samples were determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS (Malvern Instruments, Herrenberg, Germany) with a detection angle of 173°, equiped with a MPT-2 Autotitrator (Malvern Instruments, Herrenberg, Germany). For data analysis, the viscosity (0.88 mPa s) and refractive index (1.33) of distilled water at 25 °C were used. All measurements were carried out at 25 °C and at least 6 replicates of each sample were taken to check repeatability. The zeta potentials of the 0.01% (w/v) polysaccharide solutions at different pH values were determined using titration curves from pH 2.5 to 7.0 (in 0.5 ± 0.2 steps) by adding 0.25 M NaOH or 0.5 M HCl. The curves were evaluated in triplicate. The quantity of particles was considered proportional to the sample count rates (Mao et al., 2006; Sun et al., 2008).

2.4. Particle stability

The zeta potential, size distribution and quantity of particles in the 3C:7G, 2C:8G and 1C:9G samples were evaluated immediately after mixing the polysaccharides and during storage at 25 °C for 100 h (at rest).

2.5. Statistical analysis

The significant differences ($p < 0.05$) amongst the different samples were determined by the Tukey procedure, using the software STATISTICA 5.5 (Statsoft Inc., Tulsa, USA).

3. Results

3.1. Effect of polysaccharide concentration on chitosan–gellan complexation

Since complex formation between polyelectrolytes is primarily driven by coulombic interactions, the pH of the polysaccharide solution influences its charge density and therefore the complexation mechanisms and properties of the resulting PECs (Vidal et al., 2005; Sun et al., 2008). The charge densities of the gellan and chitosan solutions at the different pH values are presented in Fig. 1.

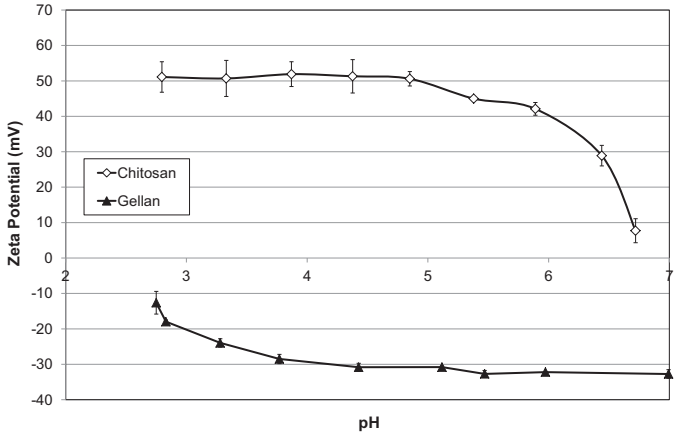

Fig. 1. Zeta potential of 0.01% (w/v) gellan and 0.01% (w/v) chitosan solutions at different pH values.

Between pH 2.8 and 5.4 the zeta potential of chitosan was statistically constant at +51 mV and decreased to +7.7 mV as the pH increased to 6.7. Such a reduction is associated with the closeness of the pH value to the pK_a of chitosan (6.2–7.0 depending on its degree of acetylation; Arrascue, Garcia, Horna, & Guibal, 2003). The amino groups of chitosan are deprotonated below the pK_a of the polysaccharide, which confers a cationic character to the chitosan.

The gellan gum was negatively charged throughout the whole pH range studied. A decrease in zeta potential was observed between pH 2.7 and 4.0, and from this point the zeta potential remained constant at −31 mV. Gellan gum is an anionic polysaccharide because of its low pK_a (3.5) which is determined by gluconic acid, a weak acid present in the gellan structure (de Jong & van de Velde, 2007). At low pH values the anionic character of gellan is reduced, due to dissociation of the carboxylic groups of gluconic acid (Horinaka, Kani, Hori, & Maeda, 2004).

To form PEC, both polymers have to be ionized and bear opposite charges (Berger, Reist, Mayer, Felt, & Gurny, 2004), since the reaction can only proceed at pH values in the vicinity of the pK_a interval of the two polymers. Therefore the systems were prepared at pH 4.5.

Table 2 shows the zeta potential of each system. Chitosan concentrations higher than 0.002% (w/v) resulted in systems with a positive zeta potential, suggesting that the amount of gellan was not enough to neutralize all the chitosan amino groups. At a chitosan concentration below 0.002% (w/v), the opposite behavior was observed, the samples showing a negative character due to the excess of gellan. The 2C:8G samples showed a zeta potential near neutrality.

The charge density of gellan gum is relatively low as compared with other anionic polysaccharides such as pectin and the carrageenans. Gellan gum has an average charge density of 0.25 mol negative charge/mol of monosaccharide in the form of a carboxylic acid group (de Jong & van de Velde, 2007). Besides the low charge density of the gellan gum, the pH of the samples (4.5) was closer to the pK_a of the gellan (3.5) than to that of the chitosan (6.5), i.e., the chitosan was more dissociated than the gellan. Other parameters that could affect the stoichiometry of complexation are the charge distribution over the polysaccharide chains, the position of the ionic groups on the chains and the chain flexibility (Hamman, 2010).

The chitosan:gellan ratio markedly affected the particle size distribution (Table 2 and Fig. 2). Chitosan (sample 100:0) showed a monomodal size distribution centered at 2.43 nm. The dimensions of chitosan depend on the semi-rigid character of the polysaccharide chains. Since chitosan is a polyelectrolyte in an acid medium, these properties are influenced by the concentration of the ions and especially by the degree of acetylation (DA) (Rinaudo, 2006). Thus different persistence lengths have been described in the literature. Chitosan was reported to measure 9 nm without acetyl groups (DA = 0.0%) (Rinaudo, 2006), 4.2 nm for DA = 0.15% (Rinaudo & Domard, 1989), 22 nm for DA ~ 0.42% (Terbojevich, Cosani, Conio, Marsano, & Bianchi, 1991) and 12.5 nm for DA = 60% (Rinaudo, 2006).

The gellan gum also presented a monomodal distribution (Fig. 2) centered at 3.15 nm. A similar value was found by Okamoto, Kubota, and Kuwahara (1993), who found a diameter of 2.4 nm.

The peaks related to the pure polysaccharides (between 1 and 10 nm) were not observed in any of the chitosan:gellan samples, suggesting that all the polysaccharide molecules were aggregated (Fig. 2).

The systems composed of both polysaccharides showed a bimodal distribution, except for the sample 2C:8G (Fig. 2). Overall, the first peak was observed between 10 and 100 nm followed by a second one between 100 and 1000 nm. An increase in the amount of gellan in the ratio enhanced the number of particles between 100 and 1000 nm and decreased the number of particles between 10 and 100 nm (Table 2). This fact suggests the existence of two different types of complex (Fig. 2). Despite the fact that chitosan has a semi-rigid structure, its persistence length, 4.2 nm for DA = 15% (Rinaudo & Domard, 1989), is much shorter than that of gellan, 98 nm (Okamoto et al., 1993). Since chain stiffness is proportional to polymer persistence length, the chitosan chain is more flexible than the gellan helix. The illustrations shown in Fig. 2, present hypothetical PEC structures for both polysaccharides. The first peak, observed near 30 nm, is probably related to PECs formed from gellan molecules surrounded by chitosan chains, whereas in the second peak (200–300 nm), the gellan molecules bridge the chitosan chains, leading to larger aggregates.

The 2C:8G samples showed a single and relatively low polydispersed peak (Fig. 2). The zeta potential of such samples was near zero, i.e., at this polysaccharide ratio there was a balance between the positive and negative charges. Therefore, the absence of repulsive interactions in such a system resulted in aggregation of the

Table 2
Zeta potential and range of diameters of the particles formed with different chitosan:gellan ratios.

System (chitosan:gellan)	Zeta potential (mV)	Volume (%)		
		1–10 nm	10–100 nm	100–3000 nm
1C:0G	51.3 ± 4.7^{a}	99.9 ± 0.2^{a}	–	–
5C:5G	59.0 ± 3.9^{a}	–	75.7 ± 1.2^{a}	24.1 ± 1.6^{d}
4C:6G	55.5 ± 4.8^{a}	–	57.8 ± 0.7^{b}	42.4 ± 0.4^{c}
3C:7G	34.7 ± 2.1^{b}	–	4.9 ± 2.9^{d}	84.0 ± 1.8^{b}
2C:8G	1.5 ± 3.6^{d}	–	–	100.0 ± 0.0^{a}
1C:9G	−28.6 ± 2.1^{bc}	–	19.7 ± 1.4^{c}	80.2 ± 2.1^{b}
0C:1G	−24.8 ± 4.8^{c}	99.9 ± 0.1^{a}	–	–

Different letters in the same column mean significant differences ($p < 0.05$).

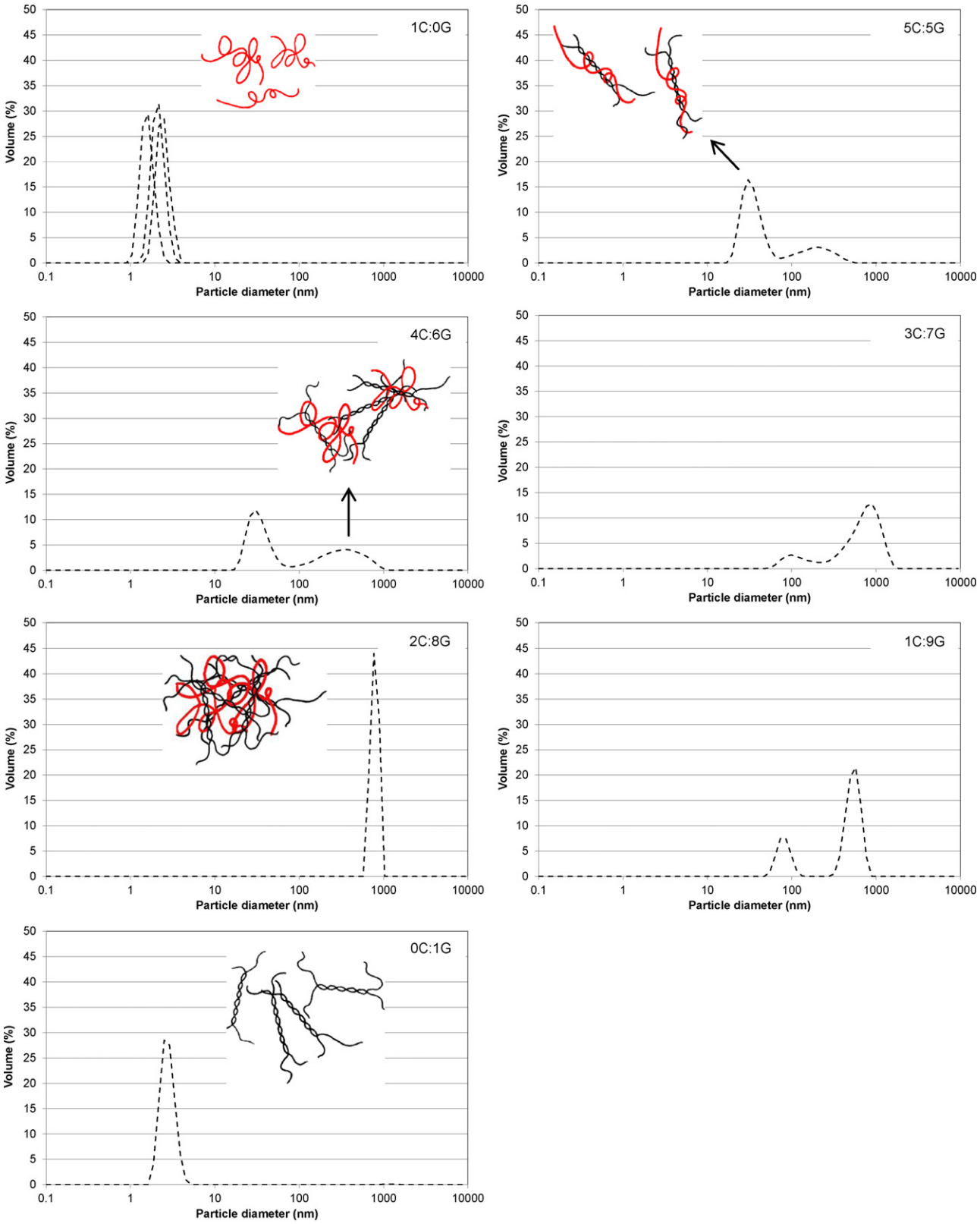

Fig. 2. Volume-weighted size distribution of chitosan (1C:0G), gellan (0C:1G) and mixed samples at different chitosan:gellan ratios. The illustrations show the hypothetical interactions between the polysaccharides.

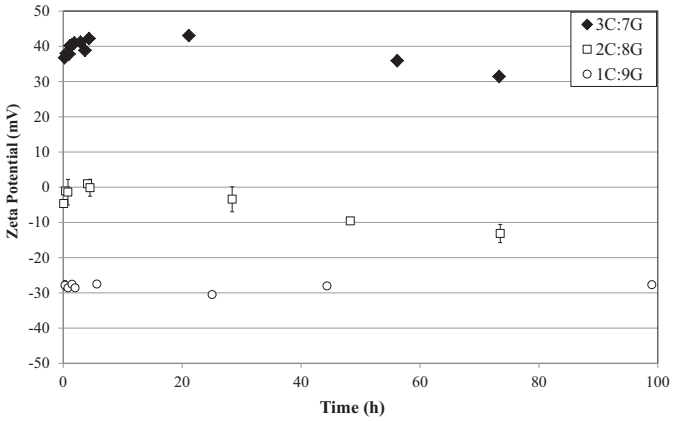

Fig. 3. Zeta potential of the samples 1C:9G, 2C:8G and 3C:7G during storage at 25 °C.

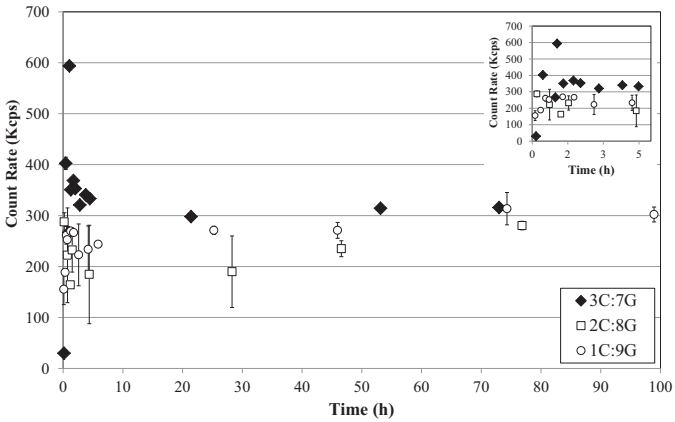

Fig. 4. Count rate of the 3C:7G, 2C:8G and 1C:9G samples during storage at 25 °C. The inset graph shows the count rate in the first 5 h after mixing the polysaccharides.

PECs. With the increase in the proportion of gellan to a 1C:9G chitosan:gellan ratio, there was an excess in the negative charge of the systems such that repulsive interactions prevailed. Again, the particles presented a bimodal size distribution (Fig. 2).

3.2. Particle stability

Overall, the PECs formed at 3C:7G, 2C:8G and 1C:9G (chitosan:gellan ratios) were stable for 100 h during storage at 25 °C, i.e., no disintegration was observed.

The changes in zeta potential, count rate and size distribution of the 1C:9G, 2C:8G and 3C:7G systems with time are presented in Figs. 4 and 5 and Table 3, respectively.

The three systems showed charge stability between 1.5 and 24 h after mixing of the polysaccharides, but different charge profiles were observed after this period (Fig. 3). The average charge of the 3C:7G samples was +40 mV in the first 24 h. From this time on, the zeta potential showed a reduction, and reached +31 mV after 73 h. The 2C:8G sample also presented a decrease in charge with time, the statistically neutral zeta potential observed up to 4 h decreasing to −13.15 mV after 73 h. This suggests that the chitosan:gellan interactions rearrange themselves with time, leading to the exposition of the negative gellan charge on the particle surface.

The only system which presented a statistically constant zeta potential with time was the 1C:9G one (Fig. 3), which also presented a constant count rate with time (Fig. 4).

The count rate is related to the number of particles in the samples (Mao et al., 2006; Sun et al., 2008). Overall, a marked variation in the count rate of the samples was observed in the first hour (Fig. 4), during which time complexation occurred simultaneously with aggregation of the PECs. The count rate of the samples 3C:7G and 1C:9G stabilized after 1.7 and 0.5 h, respectively, but the time was shorter for the 1C:9G sample due to the excess of gellan (Fig. 4), which enhanced complexation. The sample 2C:8G showed no significant statistical changes in count rate with time, since considerable aggregation occurred during storage promoted by the neutral zeta potential. This aggregation was confirmed by the low count rate values and large particle diameters (Fig. 4 and Table 2).

Therefore, the number of particles is related not only to the polysaccharide ratio but also to the aggregation rate of the complexes. An adequate polysaccharide concentration favors polyelectrolyte complexation but the neutrally charged PECs tend to aggregate, leading to larger sizes and a small number of particles, as observed in the 2C:8G samples (Figs. 3–5). In the first 24 h after sample preparation, the charge of the 2C:8G samples was statistically neutral, favoring particle aggregation (Fig. 3). After longer storage times, the structure of the 2C:8G aggregates reorganized, and gellan carboxylic groups were probably exposed on the surface of the complexes, which reduced the particle zeta potential (Fig. 3). Electrostatic interactions usually prevail in systems composed of oppositely charged polyelectrolytes (Doublier, Garnier, Renard, & Sanchez, 2000). However, the hydrophobic interactions may overlap the electrostatic interactions when the polymer presents apolar segments (Borrega, Tribet, & Audebert, 1999; Kumar, Dixit, Zhou, & Fraunhofer, 2011; Mizusaki, Morishima, & Dubin, 1998; Tsianou, Kjøniksen, Thuresson, & Nyström, 1999). In chitosan:gellan systems, the long range electrostatic interactions overcome other interactions in the early hours after mixing the polysaccharides, and are responsible for PEC formation. After charge screening, the electrostatic repulsion is reduced and, consequently, so are the

Table 3
Range in diameter of the chitosan:gellan particles with storage time at 25 °C.

Size range (nm)	Time (h)	Volume (%)		
		3C:7G	2C:8G	1C:9G
1–10	2	–	–	–
	24	–	–	–
	48	–	–*	–*
10–100	2	4.9 ± 2.9^{Bd}	–	19.7 ± 1.4^{Ad}
	24	13.6 ± 1.8^{Bc}	–	29.6 ± 2.3^{Ac}
	48	50.3 ± 2.4^{Ab}	–*	–*
100–3000	2	84.0 ± 3.4^{Ba}	100.0 ± 0.0^{Aa}	80.2 ± 1.8^{Ba}
	24	86.0 ± 1.2^{Ba}	100.0 ± 0.0^{Aa}	70.3 ± 1.2^{Cb}
	48	49.5 ± 1.5^{Ab}	–*	–*

* Above the measuring range of the equipment (6 μm).
Different capital letters in the same row and small letters in the same column mean statistically significant differences ($p < 0.05$).

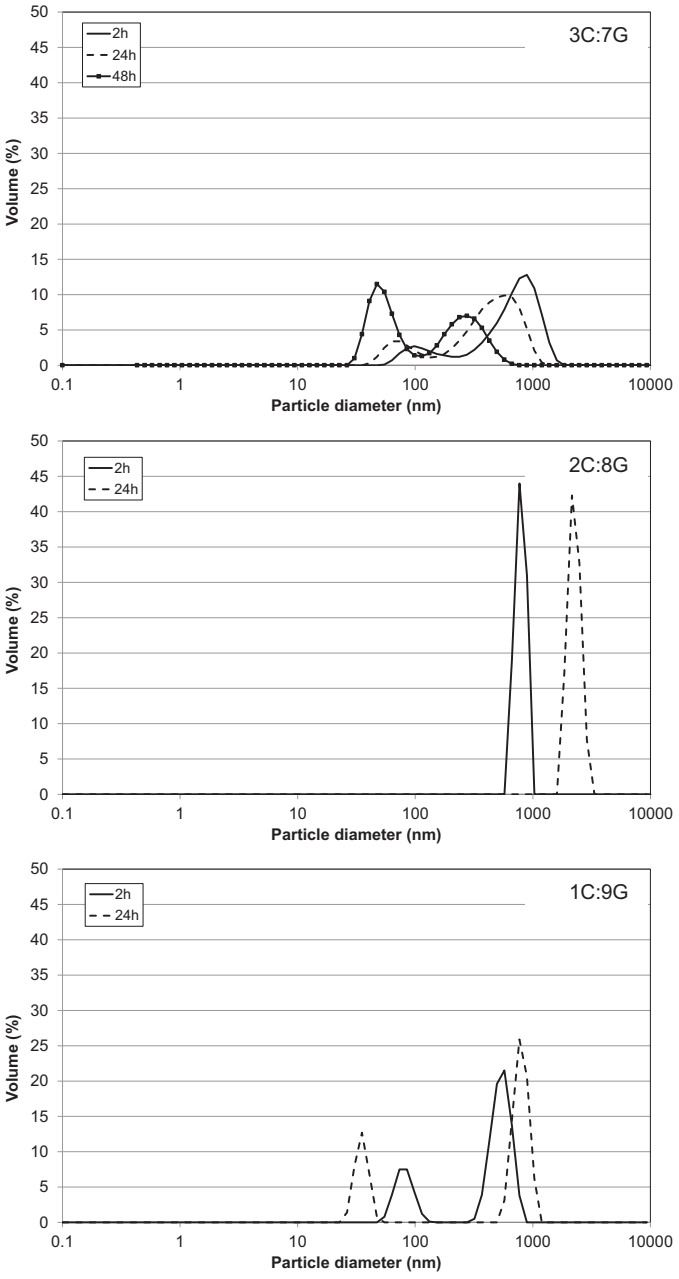

Fig. 5. Volume-weighted size distribution of the 3C:7G, 2C:8G and 1C:9G samples during storage at 25 °C.

intermolecular distances. Thus, short range interactions, such as hydrophobic interactions, are favored with time, and may cause molecular reorganization. The zeta potential results (Fig. 3) suggested that reorganization of the chitosan:gellan systems with time resulted in protection of the chitosan hydrophobic groups in the PEC core, and hence the gellan became more exposed on the PEC surface leading to a decrease in zeta potential after 48 h (Fig. 3). This reorganization was more intense in the 2C:8G system since the zeta potential of these samples was neutral in the early hours after PEC formation. In the 1C:9G systems, the structural reorganization did not affect the zeta potential, since the gellan molecules were already the major constituent on the PEC surface, due to their greater concentration with respect to chitosan.

Despite the decrease in the 2C:8G zeta potential, PEC size was enhanced with time (Figs. 4 and 6), indicating that the charge changes were not sufficient to prevent aggregation of the complexes. The size distribution in these samples (Fig. 5) showed that the particles doubled in size after 24 h. It was not possible to measure the particle size after 48 h, because the particles were larger than 6 μm (above the measuring range of the equipment). The same tendency was observed for the 1C:9G samples, but 3C:7G showed the opposite behavior. During storage at 25 °C the 3C:7G samples decreased in size. The same bimodal distribution was observed but the number of particles between 10 and 100 nm increased considerably, whilst the number of particles between 100 and 3000 nm decreased (Table 3 and Fig. 5), although the count rate remained constant (Fig. 4). This suggests that desaggregation had not occurred. In fact, the decrease in size may be related to PEC maturation. In this case the reorganization of structure of the complexes may have intensified the electrostatic interactions, which led to PEC shrinkage.

3.3. *Effect of PEC preparation on its properties*

The effect of sample preparation on the properties of the PECs was studied in uncharged (2C:8G) and charged (1C:9G) systems. As described in Section 2.2, the final concentration of the M2C:8G and M1C:9G samples (prepared in two mixing steps) matched the concentration of the 2C:8G and 1C:9G samples (prepared by the traditional mixing), respectively.

Table 4 shows the count rate and zeta potential during each preparation step of the multilayered systems, i.e., the characteristics of the M2C:8G system during mixing step 1 were the same as for system 1C:9G, prepared by traditional mixing (all together). Although no statistical changes were observed in the count rate of the samples between the first and second mixing steps, they showed different tendencies and zeta potential values (Table 4). Moreover, the samples prepared in two steps presented a marked

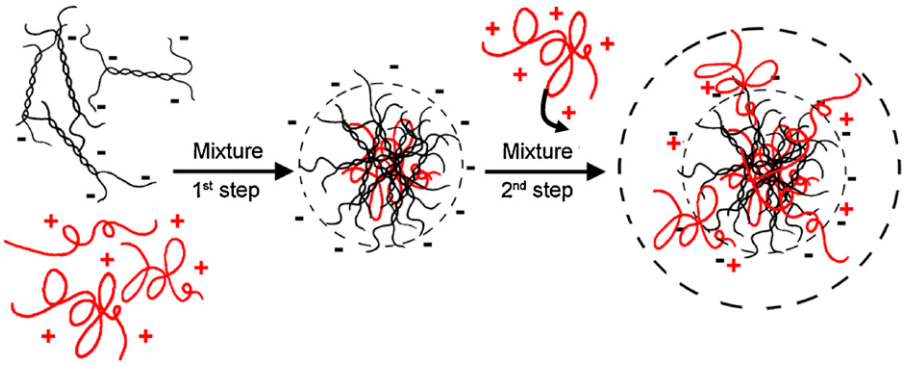

Fig. 6. Ilustration of PEC formation in the M2C:8G sample.

Table 4
Count rate and zeta potential of the samples prepared in 2 steps: properties of the samples during the first and second mixing steps.

Multilayered systems	Mixture step	Count rate (kcps)	Zeta potential (mV)
M2C:8G	1	233.6 ± 46^{ab}	-28.6 ± 2.1^{c}
	2	178.2 ± 61^{b}	-3.1 ± 0.5^{b}
M1C:9G	1	196.1 ± 92^{ab}	1.4 ± 0.7^{a}
	2	262.6 ± 17^{a}	-33.0 ± 3.5^{d}

Different letters in the same column mean statistically significant differences ($p < 0.05$).

increase in size when compared to those prepared in a single mixing step (data not shown). The final particle diameters of the samples prepared in two steps were above the limit of the equipment (6 μm), whilst all the samples prepared in one step were smaller than 3000 nm.

Besides the increase in size, the sample M2C:8G showed slightly lower values for the final count rate (178.2 kcps) and zeta potential (−3.1 mV) than the samples prepared in a single step (which showed 196.1 kcps and 1.4 mV, respectively) (Table 4). As expected, in the first preparation step the zeta potential of the M2C:8G samples was negative (−28.6 mV), which prevented particle aggregation, leading to greater count rate values (233.6 kcps). With the addition of more chitosan in the second mixing step, the negative charge decreased to −3.1 mV, and the electrostatic repulsion between the particles decreased favoring particle aggregation, which was confirmed by the slight decrease in count rate value. Particle aggregation led to a size increase, but the increase was much greater than expected from the count rate values. As previously commented, the diameter of such particles was more than two times that of the same particles prepared in a single step. This confirms that the sample preparation does affect their properties.

After the first mixing step, the surface of the M2C:8G PECs was formed mainly by gellan molecules, which conferred a negative zeta potential to the particles. In the second mixing step more chitosan was added in the system, and this cationic polysaccharide was attracted to the PEC surface, forming a multilayered particle. As both the gellan and chitosan present relatively stiff structures (Okamoto et al., 1993; Rinaudo & Domard, 1989) and were already bound during the first step, polysaccharide mobility was lower in the second step, and hence the particles showed a considerable increase in size (Fig. 6).

The M1C:9G samples presented a nearly neutral charge in the first mixing step and a low count rate due to particle aggregation (Table 4). With the addition of more gellan the particle adquired a negative charge, which led to partial disaggregation and a slight increase in count rate. A considerable increase in particle size was observed once again, suggesting that the aggregation in the second mixing step formed a multilayered particle.

3.4. Influence of surfactant

The influence of polysorbate-20 on chitosan:gellan systems was studied for the 3C:7G and 2C:8G samples. Overall, the samples with the addition of 0.0001% (w/v) polysorbate-20 showed no significant changes in size (Fig. 7), zeta potential or count rate (results not shown) during 48 h. Thus the effect of the surfactant concentration on the count rate and particle size of systems was evaluated 2 h after their preparation (Fig. 8).

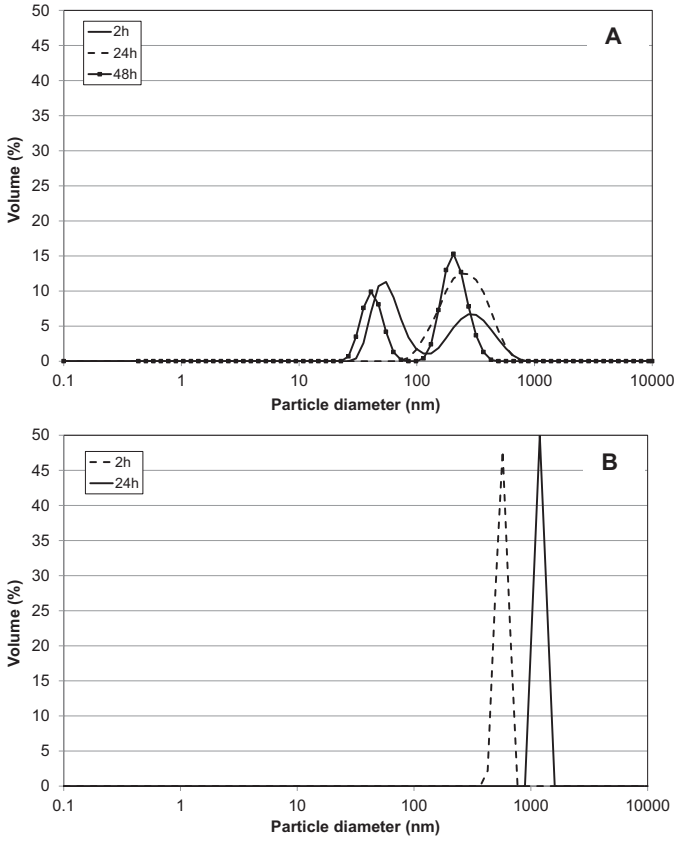

Fig. 7. Volume-weighted size distribution of the 3C:7G and 2C:8G samples with added 0.0001% (w/v) polysorbate-20 during 48 h of storage at 25 °C. (A) 3C:7G:0.1S and (B) 2C:8G:0.1S.

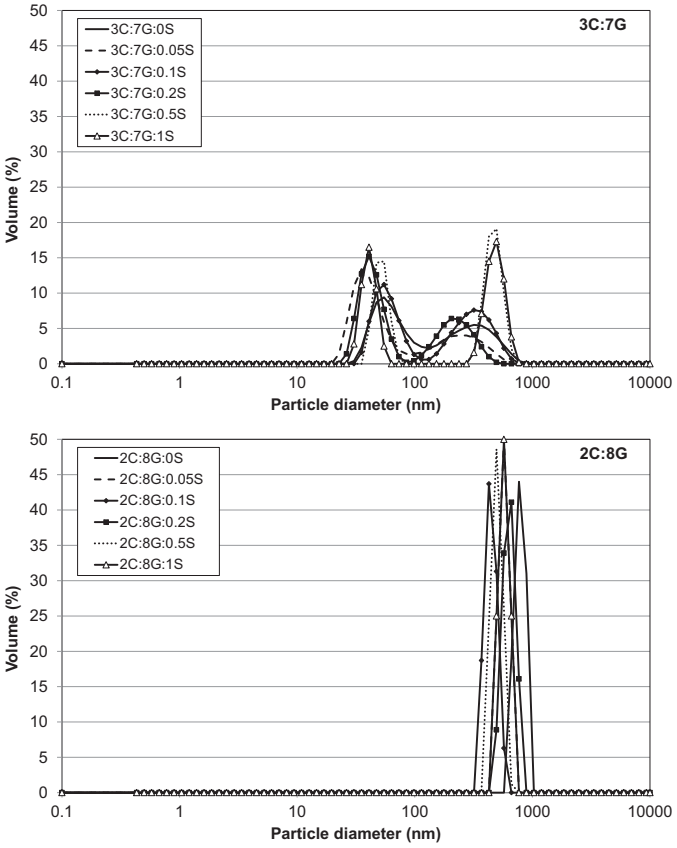

Fig. 8. Volume-weighted size distribution of the 3C:7G and 2C:8G samples with added polysorbate-20: 0.00005, 0.0001, 0.0002, 0.0005 and 0.0010% (w/v).

The presence of surfactants may change the behavior of a polymer in solution (Silva, Antunes, Sousa, Valente, & Pais, 2011). This can be exemplified by surfactant-induced thickening (Antunes, Marques, Miguel, & Lindman, 2009; Barreiro-Iglesias, Alvarez-Lorenzo, & Concheiro, 2003a), surfactant-induced swelling (Barreiro-Iglesias, Alvarez-Lorenzo, & Concheiro, 2003b) or compaction (Dias, Pais, Miguel, & Lindman, 2004), and surfactant-induced phase separation (Holmberg, Jönsson, Kronberg, & Lindman, 2003), amongst other effects. However, in the present study the count rate of the samples was statistically constant independent of surfactant concentration (data not shown). The size distribution also showed no significant changes (Fig. 8), except for a slight reduction in sample polydispersity at surfactant concentrations above 0.0005% (w/v).

Chitosan–nonionic surfactant interactions are known to be of a weak nature. The chitosan–sorbitan ester for example may interact by hydrogen bonding between the hydroxyl and carbonyl groups of the sorbitan ester head group and the amine, ammonium ions and hydroxyl groups of the chitosan, in addition to hydrophobic interactions between the sorbitan ester tails and chitosan hydrophobic sites (Grant et al., 2008). Similar interactions are expected for chitosan–polysorbate complexes. However, in the presence of an oppositely charged polysaccharide such interactions were suppressed, due to its weak nature as compared to the strong electrostatic attraction between the two oppositely charged polyelectrolytes. At high surfactant concentrations, the hydrophobic interactions between the polysorbate tail and the chitosan hydrophobic sites probably enhanced PEC shrinkage, which resulted in a decrease in sample polydispersity. Nonetheless, no significant improvement in PEC formation and stability was achieved by the addition of surfactant.

4. Conclusion

Chitosan:gellan interactions led to nanoscale PECs of different sizes. Depending on the polysaccharide ratio, the particles presented different charge characteristics, which determined particle aggregation and hence the final PEC diameter. Samples composed of 2C:8G and 3C:7G (chitosan:gellan ratio) exhibited structural reorganization during storage at 25 °C, which reduced the zeta potential of the samples. The mixing protocol was essential to determine PEC size. The multilayered particle was considerably larger than the one formed in the traditional way (1-step mixing). However, no significant changes in the PEC characteristics were observed in the presence of a non-ionic surfactant, since the driving force of PEC formation (electrostatic forces) was markedly higher than the hydrophobic and hydrogen bonding of the surfactant–polysaccharide interactions. The results presented in this paper show that it is possible to control the characteristics of chitosan:gellan PECs by changing the polysaccharide ratio and mixing methodology.

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