



# Influence of concentration, ionic strength and pH on zeta potential and mean hydrodynamic diameter of edible polysaccharide solutions envisaged for multilayered films production

Maria G. Carneiro-da-Cunha<sup>a</sup>, Miguel A. Cerqueira<sup>b</sup>, Bartolomeu W.S. Souza<sup>b</sup>,  
José A. Teixeira<sup>b</sup>, António A. Vicente<sup>b,\*</sup>

<sup>a</sup> Biochemistry Department and Laboratory of Immunopathology Keizo Asami-LIKA, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego s/n, CEP 50.670-420 Recife, PE, Brazil

<sup>b</sup> IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

## ARTICLE INFO

### Article history:

Received 4 November 2010

Received in revised form 27 February 2011

Accepted 1 March 2011

Available online 5 March 2011

### Keywords:

Polysaccharides

Zeta potential

Hydrodynamic diameter

## ABSTRACT

Polysaccharide multilayers are becoming a new and general means of modifying and functionalizing surfaces of several materials. This is achieved through changing the interfacial properties of those surfaces followed by sequential adsorption of e.g., polysaccharides. Knowledge of properties of polysaccharide solutions such as zeta potential ( $Z_p$ ) and mean hydrodynamic diameter ( $Z$ -average) is crucial to obtain stable, functional nanostructures.

In this work sodium alginate, carragennan, chitosan, and two galactomannans were characterized in terms of  $Z_p$  and  $Z$ -average, as a function of polysaccharides and NaCl concentrations and pH. The most relevant effects of these factors on  $Z_p$  and on  $Z$ -average were analysed using a  $2^3$  full factorial design.

With a few exceptions, the effect of independent variables on  $Z_p$  and  $Z$ -average values is statistically significant. In general (except for k-carragennan solutions) higher polysaccharide concentrations lead to higher  $Z$ -average values; and higher pH values lead to higher  $Z$ -average values (except for solutions of chitosan and galactomannan of *Gleditsia triacanthos*). NaCl addition decreases  $Z$ -average. For the galactomannans solutions  $Z_p$  values were found to be neutral (ranging from  $-13.7$  to  $-2.1$  mV) independently of the combinations of variables used.

This work establishes a relationship between important polysaccharides properties and the values of pH, polysaccharide and salt concentrations, thus providing insight on how to control the structure and texture of multilayered films in view of their potential application e.g., in the food, pharmaceutical, and cosmetic industries.

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## 1. Introduction

In the last years it has been shown that the sequential polysaccharide adsorption approach can be used to manipulate many different types of materials (Abu-Sharkh, 2006; Jayaraman, Hsu, & McCarthy, 2007; Yoo, Shiratori, & Rubner, 1998). Multilayer films or coatings can be made by successive adsorption of oppositely charged polyelectrolytes on a solid carrier providing potential applications on pharmaceutical, cosmetic, food science, biotechnology, paper technologies, and tissues engineering such as e.g., films for food preservation and coatings for implant devices (Crouzier, Boudou, & Picart, 2010; Elsabee, Abdou, Nagy, & Eweis, 2008; Richert et al., 2004; Rudra, Dave, & Haynie, 2006). The utilization of polysaccharides for the multilayer construction appears to be

one of the future trends in biomaterials science (Crouzier et al., 2010). Polysaccharides may be constituted either by polycations or polyanions, depending on their functional group type and can be neutral. Decher, Hong, and Schmitt (1992) introduced the electrostatic procedure of polyelectrolyte multilayer film formation. It can be applied to charged surfaces of any size and shape, and it appears to be an efficient method for obtaining various materials with defined interfacial properties. The combination of different polyelectrolytes in charged objects extends the application where these materials can be used, being of special importance in the biomaterial area (Kolasinska, Zembala, Krasowska, & Warszyńska, 2008). The layer-by-layer (LbL) assembly of noncharged organic molecules without necessary functional groups into multilayer films remains a challenge (Liu, Zhou, Geng, & Sun, 2008). However, some studies have shown that non-charged polysaccharides can also be assembled into multilayer films through halogen-bonding (Wang, Ma, Chen, Wang, & Wang, 2007), hydrogen bonding (Stockton & Rubner, 1997), coordination bonding (Xiong, Cheng, Zhou, Zhang, & Shen,

\* Corresponding author. Tel.: +351 253604419; fax: +351 253678986.  
E-mail address: [avicente@deb.uminho.pt](mailto:avicente@deb.uminho.pt) (A.A. Vicente).

1998), and charge-transfer interactions (Shimazaki, Mitsuishi, Ito, & Yamamoto, 1997). Composition of polysaccharide solutions, pH, and salt concentration are important parameters governing the formation of multilayer films. So, the understanding of polysaccharides' behaviour in different solutions is important to the process of multilayer formulation. The charge of the polysaccharides and their hydrodynamic diameter provide crucial information on how these polysaccharides will behave during the process of polyelectrolyte multilayer construction (e.g., influencing film thickness). This is determinant in the choice of the materials used and in the establishment of the experimental conditions.

One of the most used polysaccharide in the multilayer construction is chitosan (Caia, Rechtenbach, Hao, Bossert, & Jandt, 2005; Elsabee et al., 2008; Richert et al., 2004). Chitosan is a natural cationic polysaccharide formed by the N-deacetylation of chitin, a product found e.g., in crustacean shells; it is formed by a linear binary copolymer that consists of  $\beta$  (1 $\rightarrow$ 4)-linked 2-acetoamido-2-deoxy- $\beta$ -D-glucopyranose (Glc-NAc; A-unit) and 2-amino-2-deoxy- $\beta$ -D-glucopyranose (GlcN; D-unit). Due to its nontoxic, biodegradable and biocompatible properties, it has been used in the biomedical area in the form of sutures, wound healing material and for sustained release of drugs (Kumar, 2000).

Alginate is a natural anionic polysaccharide which can be extracted from marine brown algae; it is a linear binary copolymer that consists of (1 $\rightarrow$ 4)-linked  $\beta$ -D-mannuronic acid and  $\alpha$ -L-guluronic acid residues. Sodium alginate is the sodium salt of alginic acid. When extracted from the cell walls of brown algae it forms a gum that is used by food industry to increase viscosity and as an emulsifier. It is also used in indigestion tablets and on the preparation of dental impressions. A major application for sodium alginate is as thickener for reactive dyestuffs in textile screen-printing and carpet jet-printing, and it is used as a food thickener and stabilizer and also in medicines, paint, and paper coating (Izydorczyk, Cui & Wang, 2005).

Carragennan comprises a group of linear sulphated polysaccharides extracted from red seaweeds, with a primary structure based on an alternating disaccharide repeating unit of  $\alpha$ -(1 $\rightarrow$ 3)-D-galactose-4-sulphate and  $\beta$ -(1 $\rightarrow$ 4) 3,6-anhydro-D-galactose (AG) being the structures classified into three main commercial types based on the presence of the 3,6-anhydro-D-galactose on the 4-linked residue and the position and number of sulphate groups: kappa ( $\kappa$ ), iota ( $\iota$ ), and lambda ( $\lambda$ ). They are commonly used as thickener, gelling agent, and stabilizer in the food and pharmaceutical industries (Bourgoin, Zabackis, & Poli, 2008), and have been successfully used as colloidal stabilizer in the synthesis of magnetic iron oxide nanoparticles, preventing the spontaneous agglomeration of the nanoparticles (Daniel-da-Silva, Trindade, Goodfellow, Costa, Correia, & Gil, 2007).

Galactomannans are present in the endosperm of numerous plants, particularly the *Leguminosae* and are polysaccharides built up of a  $\beta$ -(1 $\rightarrow$ 4)-D-mannan backbone with single D-galactose branches linked  $\alpha$ -(1 $\rightarrow$ 6). Their mannose/galactose (M/G) ratios differ according to the species (Kök, Hill, & Mitchell, 1999) and can often be used in different forms for human consumption. Featuring different physicochemical properties, galactomannans are a versatile material used for many applications: they are excellent stiffeners and stabilizers of emulsions, and the absence of toxicity allows their use in the textile, pharmaceutical, biomedical, cosmetics and food industries (Srivastava & Kapoor, 2005; Vieira, Mendes, Gallão, & de Brito, 2007).

Light scattering techniques are particularly useful tools for exploring homo- or hetero-association of proteins and other biological macromolecules. With this technique it is possible to change solution conditions (e.g., pH, ionic strength and concentration); it is a non-invasive method where the alteration of the solution association properties does not occur, and no dilution or fractionation

of the sample that might alter the association is needed (Murphy, 1997).

In this work, zeta potential ( $Z_p$ ) and the mean hydrodynamic diameter ( $Z$ -average) of five polysaccharides have been determined under different concentrations, pH and ionic strength, thus generating a set of crucial information for their application as multilayer nanostructures for food, pharmaceutical and cosmetic industries.

## 2. Materials and methods

### 2.1. Material

The used polysaccharides were: chitosan powder at 90% deacetylation (Aqua Premier Co., Ltd.; Thailand); sodium alginate (Manutex RSX, Kelco International, Ltd.); k-carrageenan DX5253 (FMC; Belgium), and galactomannans obtained from seeds of *Gleditsia triacanthos* (collected in Botanic Garden, in Oporto, Portugal, in 2006) and *Caesalpinia pulcherrima* (collected in the Federal University of Ceará, Campus of Pici, Fortaleza; Ce – Brazil, in 2006). The galactomannan extraction was performed as described by Cerqueira et al. (2009). Lactic acid, sodium hydroxide and sodium chloride were purchased from Merck (Germany) and hydrochloric acid at 37% was obtained from Riedel-de-Haën (Germany). All other reagents were of analytical grade and used without further purification.

### 2.2. Preparation of polysaccharide solutions

The solutions were prepared dissolving the polysaccharides in distilled water (Aquatron, Bibby, England) or in 1.0% (v/v) lactic acid solution for chitosan with agitation using a magnetic stirrer (at 200 rpm) during 2 h at room temperature (20 °C). The pH was adjusted by addition of 1 M HCl or 1 M NaOH. The NaCl was added at the required concentrations. Table 1 summarizes polysaccharide and NaCl concentrations and pH values used, as established by the factorial design scheme.

### 2.3. Design of experiments

A 2<sup>3</sup> full factorial design was used to study the effects and interactions of independent variables, polysaccharide concentration, pH and salt concentration, on variables of response ( $Z$ -average and  $Z_p$ ). For each polysaccharide a set of 8 runs plus 3 replicates at the central level were performed and the levels of the independent variables (factors) under study are given in Table 1. The statistical analysis was carried out by analysis of variance (ANOVA) with  $p < 0.05$ . All statistical and graphical analyses were carried out with the "Statistica 7.0" software, 2004 edition (StatSoft Inc., Tulsa, OK, USA).

### 2.4. Zeta potential and mean hydrodynamic diameter

Zeta potential ( $Z_p$ ) and mean hydrodynamic diameter ( $Z$ -average) were determined by dynamic light scattering (DLS) with a Malvern Zetasizer, NANO ZS (Malvern Instruments Limited, UK), using a He–Ne laser (wavelength of 633 nm) and a detector angle of 173°. The DLS cumulants analysis provides the characterization of a sample through the mean hydrodynamic diameter ( $Z$ -average) for the size, determined from the intensity of scattered light. The  $Z_p$  values were calculated using the Smoluchowski equation (Hunter, 1981). Each data value is an average of three measurements.

**Table 1**  
Factor levels used in the 2<sup>3</sup> full factorial design.

Polysaccharide	Factors	Levels		
		Lower (−1)	Central (0)	Higher (+1)
Sodium alginate, galactomannan <i>CP</i> <sup>a</sup> , galactomannan <i>GT</i> <sup>b</sup> , Chitosan	Poly concentration (% (w/v))	0.2	0.4	0.6
	pH	5.0	6.0	7.0
	NaCl concentration (% (w/v))	0.0	0.05	0.1
	Poly concentration (% (w/v))	0.2	0.4	0.6
	pH	2.5	3.0	3.5
	NaCl concentration (% (w/v))	0.0	0.05	0.1
k-Carragennan	Poly concentration (% (w/v))	0.2	0.3	0.4
	pH	5.0	6.0	7.0
	NaCl concentration (% (w/v))	0.0	0.05	0.1

<sup>a</sup> Galactomannan from *Caesalpinia pulcherrima*.

<sup>b</sup> Galactomannan from *Gleditsia triacanthos*.

### 3. Results and discussion

#### 3.1. Effect of polysaccharide concentration

Polysaccharides are well known to possess a high tendency to associate, caused by the abundance in hydroxyl or amino groups present in the macromolecules that easily undergo hydrogen bonding. The reversible association occurs mostly at high concentrations and disappears again via association when the solution is diluted (Burchard, 2001). These effects can be observed in polysaccharide solutions by changing their concentration. The concentration of polyelectrolyte solutions can influence the construction of multilayers and the layers' thickness. When building multilayer systems, several works have used polysaccharide concentrations ranging between 0.1% and 0.5% (Carneiro-da-Cunha et al., 2010; Fu, Yuan, & Shen, 2005; Radeva, Kamburova, & Petkanchin, 2006; Richert et al., 2004). However, no information exists about the influence of polyelectrolyte concentration in the behaviour of the multilayer development as, for example, is found for pH and ionic strength.

Table 2 (values reproduced from Pareto charts performed on the 2<sup>3</sup> full factorial design) shows that the polysaccharide concentration was the most influent factor on *Z-average* and one of the most influent on zeta potential (*Zp*) values for all the analysed samples. These results can be explained by the occurrence of aggregation or clustering in polysaccharide solutions. The self-assembly of polysaccharide solutions can be stimulated by the hydrophobic interactions of the hydrophobic constituents resulting in a concentration-dependent increase of the size of aggregates (Camino, Pérez, & Pilosof, 2009). However, depending on the analysed polysaccharide the *Z-average* values present different ranges (Tables 3–5). This behaviour is related with the particular structure and charge of each polysaccharide.

For sodium alginate solutions the polysaccharide concentration was the unique significant factor ( $p < 0.05$ ) for *Z-average* (Table 2). In this case the increase of polysaccharide concentration leads to an increase of the *Z-average* value (Table 3). The remaining independent variables and the studied interactions between variables do not present statistically significant effects ( $p > 0.05$ ), as determined by Pareto charts (Table 2). The highest value of *Z-average* obtained for sodium alginate solution was 2155 nm for a polysaccharide concentration of 0.6%, at pH 7.0 and 0.0% of NaCl (Table 3).

In the case of chitosan solutions the increase of polysaccharide concentration leads to increasing *Z-average* values (Table 4). In this case all the independent variables and their interactions have significant effects on the *Z-average* values (Table 2). This behaviour is in agreement with the results obtained by Buhler and Rinaudo (2000) where an increase of *Z-average* values for higher values of polysaccharide concentration was reported. Also in this case the

aggregation behaviour was observed through static and dynamic light scattering; this phenomenon is related to the presence of hydroxyl and amino groups in the chitosan structure (Burchard, 2001).

Sodium alginate (0.2%) as polyanion and chitosan (0.2%) as polycation were successfully used for multilayer construction, showing that it is possible to use these two polyelectrolytes for nanomultilayer production (Carneiro-da-Cunha et al., 2010).

For galactomannan solutions the polysaccharide concentration shows a significant positive effect on *Z-average*, with a larger magnitude for galactomannan solutions of *G. triacanthos* (Table 2): increases of polysaccharide concentration lead to increasing values of *Z-average*. The highest values of *Z-average* were found to be 7467 nm and 41,000 nm for *C. pulcherrima* and *G. triacanthos* galactomannans, respectively (Table 3).

The higher values of *Z-average* obtained for galactomannan solutions can be explained by the higher molecular weight and great number of hydroxyl groups available in galactomannan structure. Neutral structures of galactomannans favour the aggregation of polysaccharide chains when compared with charged polysaccharides (alginate, chitosan and carrageenan). The *Z-average* values obtained in the present work are in the range of those reported for Arabic gum solutions that range between 200 and 8000 nm (Li, Fang, Al-Assaf, Phillips, Nishinari, & Zhang, 2009), where the increase of *Z-average* with increasing polymer concentrations was also observed. Aberle, Burchard, and Landers (2002) found values of 800 nm for aqueous Amaranth starch suspensions; and Guo, Cui, Wang, Christopher, and Young (2008) report *Z-average* values ranging between 151 and 436 nm for psyllium gum solutions (water extractable). There are differences between the two types of galactomannans studied in the present work, with the galactomannan from *G. triacanthos* displaying the highest values of *Z-average* (Table 3). This difference is possibly related with the mannose/galactose (M/G) ratio of the galactomannan from *C. pulcherrima* being higher than the M/G ratio of *G. triacanthos* galactomannan (Cerqueira et al., 2009). Picout, Ross-Murphy, Jumel and Harding (2002) presents similar results showing that locust bean gum and tara gum, with a M/G ratio of  $\approx 4$  and  $\approx 3$ , respectively, tend to form aggregates more easily than guar gum that has a M/G ratio of  $\approx 2$ . Camino et al. (2009) showed that solutions with higher molecular weight hydroxypropylmethylcellulose present higher values of *Z-average* (approximately 8000 nm).

With k-carragennan solutions the polysaccharide concentration presents a significant negative effect on *Z-average* value, followed by the significant negative effect of NaCl concentration as well (Table 2): increasing either of these two variables will provoke a decrease of the value of *Z-average* (Table 5). These results are in conformity with the values obtained by Abad et al. (2008) for a 0.5% k-carragennan solution at 40 °C. This behaviour is possibly due to

**Table 2**

Estimated effects (absolute values) of independent variables (factors) and their interactions on Z-average (Zav) and on zeta potential (Zp) obtained from the Pareto charts of effects.

Independent variables (factors) and interactions	Sodium alginate	<i>C. pulcherrima</i>	<i>G. triacanthos</i>	Chitosan	k-Carragennan
On Z-average (Zav)					
Polymer conc. (% (w/v))	30.86*	69.59*	451.00*	398.00*	−26.30*
pH	−4.05	22.11*	−321.00*	−200.45*	−0.40
NaCl conc. (% (w/v))	3.45	−5.06*	−113.00*	−69.28*	−21.80*
pH by NaCl conc.	1.99	−0.66	83.00*	64.78*	−8.30*
Polymer conc. by pH	−1.98	−27.38*	−305.00*	−75.56*	−2.40
Polymer conc. by NaCl conc.	1.18	−1.46	−119.00*	28.50*	9.00*
On zeta potential (Zp)					
Polymer conc. (% (w/v))	−13.41*	12.39*	60.90*	19.95*	−58.77*
pH	−3.53	−12.77*	38.50*	−24.51*	−23.64*
NaCl conc. (% (w/v))	10.07*	13.15*	112.80*	−8.44*	28.08*
pH by NaCl conc.	−1.18	7.83*	−37.80*	7.72*	−7.10*
Polymer conc. by pH	6.23*	0.99	−38.20*	−3.73	21.38*
Polymer conc. by NaCl conc.	7.68*	−8.02*	−20.30*	7.34*	8.14*

\* Significant effects at the 95% level.

**Table 3**

Zp and Z-average of the 2<sup>3</sup> full factorial design for sodium alginate and galactomannans of *Caesalpinia pulcherrima* and *Gleditsia triacanthos*.

Run	Polymer conc. (% (w/v))	pH	NaCl conc. (% (w/v))	Sodium alginate		<i>C. pulcherrima</i>		<i>G. triacanthos</i>	
				Zp (mV)	Z-average (nm)	Zp (mV)	Z-average (nm)	Zp (mV)	Z-average (nm)
1	0.2	5.0	0.0	−59.3	1133	−7.5	2743	−13.0	4813
2	0.2	5.0	0.1	−52.0	830	−4.5	2070	−3.8	4693
3	0.2	7.0	0.0	−63.9	1180	−13.7	4743	−6.0	3800
4	0.2	7.0	0.1	−66.5	1140	−5.6	5053	−4.4	4433
5	0.6	5.0	0.0	−82.2	2106	−2.9	7173	−5.9	41,000
6	0.6	5.0	0.1	−67.5	2013	−3.1	7403	−2.8	26,133
7	0.6	7.0	0.0	−82.2	2155	−7.6	7467	−6.6	11,170
8	0.6	7.0	0.1	−62.2	2060	−4.7	6580	−2.1	8255
9 <sup>a</sup>	0.4	6.0	0.05	−59.3	1190	−5.8	6667	−5.0	6597
10 <sup>a</sup>	0.4	6.0	0.05	−59.9	1098	−6.0	6525	−4.9	6550
11 <sup>a</sup>	0.4	6.0	0.05	−61.9	1157	−5.3	6583	−5.0	6658

Each value of Zp and Z-average is an average of three determinations.

<sup>a</sup> Central level.

**Table 4**

Results of the 2<sup>3</sup> full factorial design for chitosan. Each value of Zp and Z-average is an average of three determinations. C, central level.

Run	Polymer conc. (% (w/v))	pH	NaCl conc. % (w/v)	Zp (mV)	Z-average (nm)
1	0.2	2.5	0.0	+52.1	1848
2	0.2	2.5	0.1	+41.2	772
3	0.2	3.5	0.0	+38.4	580
4	0.2	3.5	0.1	+31.6	273
5	0.6	2.5	0.0	+63.5	5073
6	0.6	2.5	0.1	+56.3	4253
7	0.6	3.5	0.0	+41.1	2590
8	0.6	3.5	0.1	+47.1	2833
9 <sup>(C)</sup>	0.4	3.0	0.05	+60.7	2470
10 <sup>(C)</sup>	0.4	3.0	0.05	+59.7	2450
11 <sup>(C)</sup>	0.4	3.0	0.05	+59.1	2460

**Table 5**

Results of the 2<sup>3</sup> full factorial design for k-carragennan. Each value of Zp and Z-average is an average of three determinations. C, central level.

Run	Polymer conc. (% (w/v))	pH	NaCl conc. (% (w/v))	Zp (mV)	Z-average (nm)
1	0.2	5.0	0.0	−39.6	935
2	0.2	5.0	0.1	−24.9	868
3	0.2	7.0	0.0	−56.8	1273
4	0.2	7.0	0.1	−51.9	578
5	0.4	5.0	0.0	−81.5	739
6	0.4	5.0	0.1	−61.6	471
7	0.4	7.0	0.0	−80.5	596
8	0.4	7.0	0.1	−64.8	547
9 <sup>(C)</sup>	0.3	6.0	0.05	−57.8	776
10 <sup>(C)</sup>	0.3	6.0	0.05	−58.2	758
11 <sup>(C)</sup>	0.3	6.0	0.05	−59.1	741



the fact that k-carrageenan possesses a high content of 3,6 AG and may undergo syneresis.

The aggregation of the polysaccharide solutions can be related with the charge of the polysaccharide, where different charges will induce different polysaccharide behaviours. The charge of the polysaccharide has a direct relationship with the zeta potential ( $Z_p$ ) of the solution.  $Z_p$  is defined as the electrical potential which exists at the hydrodynamic plane of shear surrounding a charged particle and is essentially the potential at the point in space where low molecular weight ions cease to move with the particle and remain within the surrounding solvent (McConaughy, Stroud, Boudreaux, Hester, & McCormick, 2008). Generally, when all the particles have a large positive or negative  $Z_p$  (where the positivity and negativity is greater or lower than +30 mV and –30 mV), they will repel each other and the dispersion is stable. On the other hand, when the particles have low  $Z_p$  values, there will be no sufficient force to prevent the particles from the aggregating (Wongsagonsup, Shobsngob, Oonkhanond, & Varavinit 2005).

Table 2 shows that  $Z_p$  is significantly influenced ( $p < 0.05$ ) by polysaccharide concentration for all the studied polysaccharides. Sodium alginate and k-carrageenan solutions present negative values of  $Z_p$  (Tables 3 and 5) explained by their anionic structure. In these two polysaccharides the increase of the polysaccharide concentrations leads to more negative  $Z_p$  values.

Chitosan solutions present positive values of  $Z_p$ , ranging between +31.6 and +63.5 mV (Table 4). The results are in agreement with the value obtained by Vargas, Albors, Chiralt, and González-Martínez (2009), with a  $Z_p$  of +62 mV for a solution of 1.0% chitosan, 1.0% acetic acid and 0.1% Tween 80.

Galactomannan solutions present  $Z_p$  values ranging between –13.7 and –2.1 mV, confirming their neutral behaviour (Table 3).

The type of polysaccharide used is of a major importance when building multilayered structures where weak bonds are important to e.g., control the thickness of the layers (Elzbieciak, Zapotoczny, Nowak, Krastev, Nowakowska, & Warszynski, 2009).

### 3.2. Effect of ionic strength

Different NaCl concentrations were added to the solutions aiming at evaluating the influence of ionic strength in  $Z$ -average and  $Z_p$  of polysaccharide solutions. Cations, such as  $\text{Na}^+$ , are known to affect polysaccharides' conformational properties. In addition to the practical interest, the understanding of the mechanism leading from molecular conformational change to macroscopic aggregation is, at the present time, relevant in polymeric aggregation studies (Mangione, Giacomazza, Bulone, Martorana, Cavallaro, & San Biagio, 2005). Evaluation of the effect of ionic strength in  $Z$ -average and  $Z_p$  will contribute to improve our understanding of such mechanism.

The ionic strength shows influence on  $Z$ -average for all polysaccharides with exception of sodium alginate, where no statistically significant influence ( $p > 0.05$ ) was observed (Table 2). These results can be explained by alginate's structure, which in this case was already influenced by the presence of  $\text{Na}^+$ . Strand, Bere, Dalberg, Sikkeland and Smidsrod (1982) showed that at low concentrations (0.24 g/L) of sodium alginate the ionic strength presents a small effect on the polysaccharide shape.

$Z$ -average values for both galactomannan solutions also show to be significantly influenced by ionic strength (Table 2). In all cases, with exception of runs 3 and 4, the increase of NaCl concentration leads to lower values of  $Z$ -average (Table 3). These results are in agreement with Barry and Halsey (1963) who showed that urea reduces the aggregation in aqueous solution of locust bean galactomannan. Like urea, also NaCl can act by breaking the hydrogen bonds of galactomannan solutions (Li, Jianga, Chen, Yang, & Guan, 2004). Also the interactions between NaCl and polysaccharide

concentrations, and NaCl concentration and pH, present statistically significant influences ( $p < 0.05$ ) on  $Z$ -average of *G. triacanthos* galactomannan solutions (Table 2). The effects of the interactions are different in each case: while improved interactions between NaCl and polysaccharide concentrations lead to higher values of  $Z$ -average, for improved interactions between NaCl concentration and pH such values decrease (Table 3).

Table 2 shows that  $Z$ -average values of chitosan solutions are significantly influenced by NaCl concentration. The increase of NaCl concentration leads to a decrease of the  $Z$ -average values. This phenomenon can be related with the diminution of the repulsive potential that result in the increase of flocculation or precipitation (Cho, Heuzey, Bégin, Pierre, & Carreau, 2006).

For k-carrageenan solutions an increase of the ionic strength leads to lower values of  $Z$ -average (Table 5). This behaviour can be explained by the presence of ions such as  $\text{Na}^+$  that affect the structural conformation of the carrageenan solutions (Abad et al., 2008), decreasing the aggregation among polysaccharide molecules. It was also observed that the interaction between the concentration of k-carrageenan concentration and NaCl when at their highest levels (run 6 in Table 5) led to the lowest value of  $Z$ -average (471 nm).

$Z_p$  values are in all cases significantly influenced ( $p < 0.05$ ) by NaCl concentration (Table 2). Also the interactions between NaCl and polysaccharide concentrations, and NaCl concentration and pH (with the exception of sodium alginate) have a statistically significant influence ( $p < 0.05$ ) on  $Z_p$ .

Sodium alginate and k-carrageenan solutions (featuring negative  $Z_p$  values) show a slight increase (closer to zero) of  $Z_p$  values (Tables 3 and 5) when NaCl concentration increases. Table 3 shows that for galactomannan solutions (also with negative  $Z_p$  values), a pH increase leads to lower  $Z_p$  values, being pH a statistically significant factor. Also, the increase of ionic strength (through increasing NaCl concentrations) leads to a decrease of  $Z_p$  values. These results are in agreement with McConaughy et al. (2008), who reported that the increase of ionic strength leads to lower (closer to –25 mV)  $Z_p$  values for galacturonate solutions.

In the case of chitosan solutions the increase of NaCl concentration leads to a decrease of  $Z_p$  with exception of runs 7 and 8 (Table 4). The reduction of the repulsive potential, favoured by the interaction of the charged amino groups with the anions  $\text{Cl}^-$ , is possibly the cause for this behaviour. This repulsion reduction results in an increased potential of flocculation or precipitation (Cho et al., 2006).

The ionic strength shows a great influence on  $Z_p$  values and can therefore be used to enhance the polyelectrolyte behaviour of the polysaccharides.

### 3.3. Effect of pH

Some authors state that the effect of pH is much stronger than that of ionic strength and other factors on an electrostatic self-assembly process of a weak and strong polyelectrolyte (Choi & Rubner, 2005; Fu et al., 2005; Yoo et al., 1998). Some works studied the influence of pH on multilayer construction, showing that it affects the thickness of the multilayer. It has been shown that for chitosan and sodium carboxymethylcellulose multilayer the increase of pH (from 4 to 5.5) of sodium carboxymethylcellulose decreases the multilayer thickness (Radeva et al., 2006). This behaviour was also observed for chitosan and heparin (Boddohi, Killingsworth, & Kipper, 2008).

Table 2 shows that pH is a statistically significant factor ( $p < 0.05$ ) on the  $Z$ -average of the two galactomannans and chitosan solutions. The influence of pH is negative and of particularly high magnitude on  $Z$ -average values of *G. triacanthos* galactomannan and chitosan solutions. For instance, for the same polysaccharide and NaCl concentration, the values of  $Z$ -average for chitosan solu-

tion reduce from 1848 to 580 nm when the pH increases from 2.5 to 3.5 (Table 4). Also for *G. triacanthos* galactomannan solution *Z-average* values fall from 41,000 to 11,170 when pH increases from 5.0 to 7.0 while maintaining polysaccharide and NaCl concentrations (Table 3).

No statistically significant influence ( $p > 0.05$ ) was observed for the *Z-average* values of k-carragennan and sodium alginate solutions (Table 2). Alginate and k-carragennan do not present aggregation for pH changes (within the studied range), which can be explained by the low  $pK_a$  values of these two polysaccharides 3.38–3.65 and 2.0 (Gu, Decker, & McClements, 2005; Xu, Zhan, Fan, Wang, & Zheng, 2007), respectively. These values are very far from the pH limits used in this work (Table 1). Even so, for k-carragennan solution and for similar polysaccharide and NaCl concentrations (see Table 5), the value of *Z-average* increased from 935 to 1273 nm when pH increases from 5.0 to 7.0.

*Z-average* of chitosan solutions suffers a strong influence of pH and of the interaction of pH with both polysaccharide and NaCl concentrations. The decrease of the pH value of chitosan solution from 3.5 to 2.5 provokes significant increases of *Z-average* values (Table 4). These results are in agreement with Pa and Yu (2001) who demonstrated that a decrease of pH leads to increasing values of intrinsic viscosity, suggesting a chain expansion of chitosan molecules. It is well known that the presence of acid causes the  $-NH_2$  groups present in the chitosan molecules to become positively charged  $-NH_3^+$  groups. Therefore, the amount of acid (e.g., used for pH adjustment) strongly affects the charge density of chitosan molecules and thus the chain conformation.

In the case of galactomannan solutions changes in pH affect *Z-average* in different manners. The interaction between *C. pulcherrima* galactomannan concentration and pH presents a negative effect on *Z-average* while, for pH alone, the effect is positive (Table 2). On the other hand *G. triacanthos* galactomannan solutions have pH has the second most influent factor affecting *Z-average*. The observed behaviour of *Z-average* upon variation of pH can be related with the electrostatic repulsion of the molecules in solution that changes with pH modification (Wang & Cui, 2005).

Table 2 shows that pH is one of the most influent factors ( $p < 0.05$ ) on the values of *Zp*, with the exception of sodium alginate solutions.

The galactomannan of *G. triacanthos* presents the lowest value of *Zp* (−13 mV) for a polysaccharide concentration of 0.2%, at pH 5.0 and no NaCl. The galactomannan of *C. pulcherrima* shows the lowest value of *Zp* (−13.7 mV) at a polysaccharide concentration of 0.2%, at pH 7.0 and no salt (Table 3).

For chitosan solutions the increase of pH lead to a decrease of the *Zp* for similar salt and polysaccharide concentrations (Table 4), possibly resulting from a decrease of the charge density of the polymer provoked by the reduction of the concentration of  $H^+$  ions. In the work of Boddohi et al. (2008) a similar result has been reported, where a decrease of pH lead to an increase of the charge density of the chitosan chain.

#### 4. Conclusion

Multilayer development can be influenced by the material and by the used conditions in their construction. Results presented in this work showed that polysaccharide concentration, pH and the ionic strength are influent factors in *Z-average* and *Zp* values. Also the interactions between independent factors are reported and showed to have influence in the solutions properties.

All disclosed information is a useful contribution to understand polysaccharides' properties and how these properties can be managed to control the structure and texture of manufactured products

in view of their potential application in the food, pharmaceutical, and cosmetic industry.

#### Acknowledgments

The author M.G. Carneiro-da-Cunha is recipient of a fellowship from Erasmus Mundus External Cooperation Window (lot 17) – EMUNDUS17 and also gratefully acknowledges financial support from CAPES/Procad/1415/2007. M.A. Cerqueira is recipient of a fellowship from Fundação para a Ciência e Tecnologia (FCT, SFRH/BD/23897/2005) and B.W.S. Souza is a recipient of a fellowship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil (Capes, Brazil).

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