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ORIGINAL ARTICLE

Complex coacervates between bovine serum albumin and anionic polysaccharides: formation and characterization

Complexos coacervados entre albumina do soro bovino e polissacarídeos aniônicos: formação e caracterização

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Abstract

The comparative study regarding complexes coacervated between Bovine Serum Albumin (BSA) and different polysaccharides, Pectin (PEC) and Gum Acacia (GA), was carried out by evaluating the influence of different ratios (protein:polysaccharide) and sodium chloride (NaCl) concentrations on turbidity and zeta potential. The BSA:PEC complexes were formed in a 10:1 ratio whereas BSA:GA at 3:1. The complexation pH showed different behavior, BSA: PEC complexes exhibited maximum turbidity in a wide pH range (4.9 to 1.5), while BSA: GA had maximum turbidity at pH 3.5. The increase in the concentration of NaCl negatively influenced the complexation. The NaCl concentration of 0.40 mol L^{-1} suppressed the interaction in BSA:PEC (10:1) and reduced the range formation of BSA:GA (3:1). The Fourier Transform Infrared (FTIR) demonstrated the participation not only of electrostatic interactions, but also of hydrogen bonds in the complexation. This initial study elucidated fundamental aspects about the formation of coacervate complexes between BSA:GA/PEC that assist in directing its application in food products especially, in acidic matrices (pH~4.0) as well as with low concentration of salts, in view of the effect of pH on maximum formation and sensitivity to NaCl. These complexes can be added directly to products in order to add nutritional value or even be used as a new matrix for the encapsulation of bioactive compounds.

Keywords: Gum acacia; Pectin; Whey protein; BSA; FTIR; Encapsulation.

Resumo

O estudo comparativo de complexos coacervados entre albumina sérica bovina (BSA) e diferentes polissacarídeos, pectina (PEC) e goma acácia (GA), foi realizado avaliando-se a influência de diferentes razões (proteína:polissacarídeo) e concentrações de cloreto de sódio (NaCl) na turbidez e no potencial zeta. Os complexos BSA:PEC foram formados na proporção 10:1, enquanto os de BSA:GA, na proporção 3:1. O pH de complexação apresentou comportamento

diferenciado, complexos BSA:PEC exibiram máxima turbidez em uma ampla faixa de pH (4,9 a 1,5), enquanto complexos BSA:GA tiveram turbidez máxima em pH 3,5. O aumento da concentração de NaCl influenciou negativamente a complexação, com a concentração de NaCl de 0,40 mol L^{-1} capaz de suprimir a interação em BSA:PEC (10:1) e reduzir a faixa de formação de BSA:GA (3:1). A espectroscopia de infravermelho com transformada de Fourier (FTIR) demonstrou a participação não apenas de interações eletrostáticas, mas também de ligações de hidrogênio na complexação. Este estudo inicial elucidou aspectos fundamentais sobre a formação de complexos coacervados entre BSA:GA/PEC que ajudam a direcionar sua aplicação em produtos alimentícios, especialmente em matrizes ácidas e com baixa concentração de sais, tendo em vista o pH máximo de formação (pH ~ 4,0) e a sensibilidade ao NaCl. Esses complexos podem ser adicionados diretamente em produtos, a fim de agregar valor nutricional ou ainda serem utilizados como uma nova matriz para o encapsulamento de compostos bioativos.

Palavras-chave: Goma acácia; Pectina; Proteína de soro; BSA; FTIR; Encapsulação.

1 Introduction

The interaction between biopolymers is unstable and susceptible to the phenomenon of phase separation. This separation may occur due to thermodynamic incompatibility (segregation), as well as to the predominance of charge repulsion or complexation (complex coacervation), when there is a predominance of attraction between oppositely charged biopolymers (Souza et al., 2013; Kruif & Tuinier, 2001; Tolstoguzov, 1991). Coacervation is defined by the International Union of Pure and Applied Chemistry (1997), as being a colloidal separation of systems in two liquid phases, because after the interaction in which a liquid phase is formed, without polymers and another one rich in polymer and electrically neutral, it is stabilized mainly by electrostatic interaction. Because it occurs through opposite charges, complex coacervation is influenced by intrinsic factors, such as charge density and distribution of its reaction groups and extrinsic factors such as pH, temperature, ionic strength, proportion and concentration of biopolymer (Souza et al., 2013; Kruif & Tuinier, 2001).

Complex coacervates have potential applications in the food and pharmaceutical industry, such as film making (Silva et al., 2018), emulsion stabilization (Bago Rodriguez et al., 2018) and protein purification (Souza et al., 2018b; Yang et al., 2020). However, the main application of coacervate complexes is related to the encapsulation and controlled delivery of bioactive compounds sensitive to environmental conditions (Eghbal & Choudhary, 2018; Santos et al., 2021). The technique stands out for its high encapsulation efficiency (up to 99%), low processing cost, and for not using chemical reagents and high temperatures (Souza et al., 2013; Timilsena et al., 2019). However, in order for these complexes to be applied in food systems, a detailed study of their behavior in relation to the variation of pH, temperature and salt concentration is necessary (Dickinson, 2008).

Coacervate complexes can be obtained from numerous polyelectrolytes, mainly biopolymers such as proteins and polysaccharides, as they are biocompatible, biodegradable and non-toxic (Silva & Andrade, 2009). Whey is an excellent source of protein, although it is part of the scenario of biofunctional products and of high nutritional value, it has still been discarded by some dairy industries (Ganju & Gogate, 2017). One of the most well-characterized whey proteins is Bovine Serum Albumin (BSA), globular, consisting of 582 amino acid residues, 66.62 kDa molar mass and isoelectric point of 4.7 (Zhao et al., 2009)

Among the polysaccharides used in the coacervation process are Pectin (PEC) and Gum Acacia (GA). The first one is associated with a structural polysaccharide that confers rigidity to the cell wall of vegetables being used in the food industry for its gelling, thickening and stabilizing properties. It differs by the degree of esterification being low methyl (< 50%) and high methyl (> 50%). The degree of esterification influences the structure, interaction, and properties of PEC. It has pKa between 2.0 ~ 3.0 and molar mass of 70 to 140 kDa (Muhoza et la., 2019). The second is related to an exudate of trees marketed for more than 5,000 years, has pKa 2.5 and molecular weight 350 kDa. It has no odor, taste, and color is considered a complex anionic

polysaccharide, because it has a protein fraction responsible for its emulsifying property (Liu et al., 2009; Islam et al., 1997; Anvari et al., 2015).

Previous studies (Vinayahan et al., 2010; Li et al., 2012; Ru et al., 2012) have demonstrated the formation of complexes between these biopolymers (BSA, PEC and GA), however, information regarding the chemical composition and chemical bonds involved in the formation of these complexes are still rare in the literature.

This study aimed to study and compare the influence of pH, ionic strength and ratio (protein:polysaccharide) on the formation of coacervate complexes obtained from BSA and PEC/GA, for later application in food products and characterize their formation through Fourier Transform Infrared spectroscopy (FTIR spectroscopy).

2 Material and methods

2.1 Materials

The PEC from citrus peel (Galacturonic acid > 74%) and degrees of esterification > 67%, GA from acacia tree and BSA (≥ 96% pure) were purchased from Sigma Chemicals (St. Louis, USA). Sodium chloride (NaCl), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from VETEC® Ltda, (Rio de Janeiro, Brazil). The water used was of ultrapure quality (Gehaka-Master P & D - Brazil), with a conductivity of 0.05 μS/cm.

2.2 Preparation of complexes

The polysaccharide concentration was set at 0.1% (w/v) and BSA varied in five ratios (1:1, 2:1, 3:1, 5:1, 10:1). To determine the effect of NaCl, five concentrations of NaCl (0.0, 0.01, 0.05, 0.1 and 0.4 mol L^{-1}) were used. The materials were weighed using an analytical balance mod.B-TEC-210ª (Tecnal, Brazil) and had an uncertainty of ± 0.0001 g. The solutions were solubilized with the aid of a magnetic stirrer NT101 (Nova Tecnica, Brazil) for three hours at room temperature (25 °C). For further turbidimetric titration, the pH of the solutions was previously adjusted to pH 7.0 with sodium hydroxide (NaOH) and with the aid of a bench pH meter mPA-210 (Tecnopon, Brazil) (Gulão et al., 2014).

2.3 Turbidimetric titration

The transmittance of the samples was measured with a UV-Vis spectrophotometer (Libra S12, Biochrom, England) at a wavelength of 400 nm using a glass cuvette with 1 cm optical path. The equipment was calibrated for 100% transmittance (T) with ultrapure water and turbidity defined as 100 - % T (Souza et al., 2013).

The solution containing the defined protein:polysaccharide ratio and its respective NaCl concentration had its pH adjusted from 7.0 to 1.5 using HCl of different concentrations (0.5, 1 and 1.5 mol L^{-1}) and NaOH (0.25 mol L^{-1}), with the aid of a magnetic stirrer and benchtop pH meter. Aliquots were then taken to measure the transmittance value. All titrations were performed at room temperature (~ 25 °C) in triplicate.

2.4 Potential – Zeta (ζ)

Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) was used to determine the potential-ζ of protein:polysaccharide ratios, and of isolated proteins and polysaccharides. Initially, samples (10 mL) at 0.1% (w/w) were added to a MPT-2 titrator (Malvern Instruments, Worcestershire, UK) containing 0.5 mol L^{-1} HCl and 0.25 NaOH solutions and 0.025 mol L^{-1}. The titration was done between pH 2.0 and 7.0, in the range of 0.5 ± 0.1. The potencial-ζ was calculated based on the mathematical model of Smoluschwsky and the analysis was performed at room temperature (~ 25 °C) in triplicate.

2.5 Fourier Transform Infrared (FTIR) spectroscopy

Infrared spectra were obtained from the BSA, PEC, GA and lyophilized complex coacervations (Terroni, Enterprise I, Brazil) at the defined ratio. The analyzes were performed using the FTIR spectrophotometer Vertex 70 (Bruker, Germany) read in the range between 4000 to 500 cm^{-1}.

3 Results and discussions

3.1 Effect of pH and ratio on the formation of coacervated complexes

The formation of the protein: polysaccharide complexes was verified by the variation of turbidity as a function of pH as well as the ratio between BSA: PEC (Figure 1a) and BSA: GA (Figure 1b). The initial pH of the solution was 7.0 and the slow addition of HCl reduced the pH to 1.5. During the titration and formation of the coacervate complexes between BSA:PEC (Figure 1a) three distinct turbidity regions, known as transition regions (pH_c, $pH_{Ø1}$ and $pH_{Ø2}$), were found. For all the studied reasons, the turbidity values remained very low, i.e., practically constant between pH 7.0 and 6.0, indicating the polymers presented in the soluble form. Between pH 6.0 and near pH 5.0 a gradual increase of the turbidity was observed, being denominated pHc. This point ($pH > pH_c$) indicates the beginning of the formation of the soluble complexes through non-covalent attractions between the polymers (Gulão et al., 2014; Weinbreck et al., 2003). These soluble complexes are formed at a pH above the isoelectric point (pI) of the protein, with the presence of weak electrostatic interaction and high net load among biopolymers (Ru et al., 2012; Jones et al, 2009). It could be found that for different reasons different pH_c are found. Possibly, this behavior is due to the equilibrium of charges between the polymers that occurs at different pHs owing to the difference of loads present in each system.

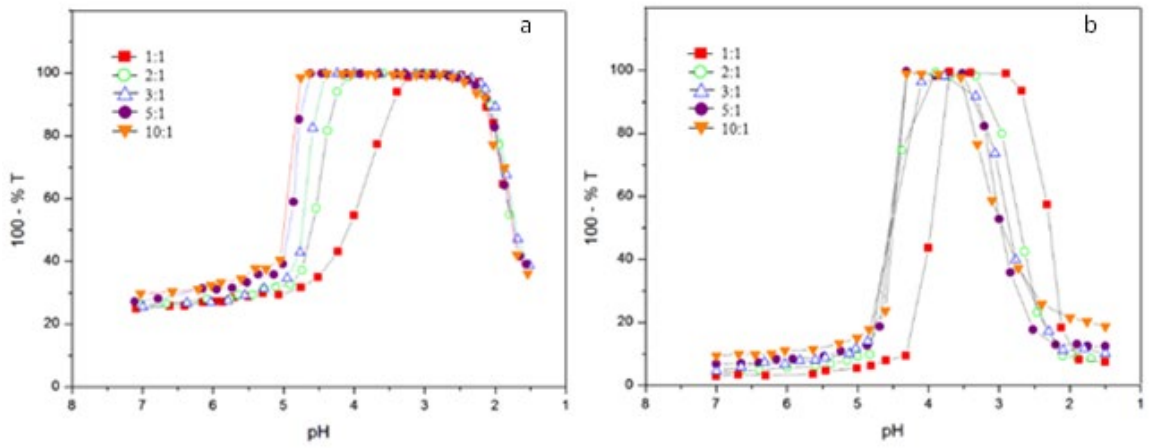

Figure 1. Turbidity (100 - T%) versus pH of a system containing (a) BSA:PEC and (b) BSA:GA in different ratios.

From pH 5.0 to pH 4.5, near the isoelectric point of BSA (~ 4.7), there was a fast increase in turbidity ($pH_{Ø1}$). This phase is characterized by the appearance of the coacervate phase, where the polysaccharide has negative charges and the protein positive charges, occurring electrostatic interaction and formation of insoluble complexes (Turgeon et al., 2003). Being close to pH 4.5, except for the ratio 1:1 that only occurred near pH 3.0, the turbidity presented its maximum value making the solution look completely cloudy. The increased turbidity indicated the formation of a seemingly stable colloidal suspension of biopolymers sufficient to promote light scattering (Jones & McClements, 2010). As observed in the results presented in Figure 1a, the pH maximum for BSA:PEC was achieved in a pH range (4.5 to 2.0), as well as also reported by Souza et al. (2018a), for complexes coacervated between PEC and lysozyme. This region represents a large interval for the application of these complexes in different food matrices.

At pH 2.0 low turbidity values indicated the beginning of the dissociation of the biopolymers and in pH 1.5, the solution became totally transparent indicating the end of the complexation, being known as $pH_{Ø2}$. At this pH, PEC (pKa 2.5 ~ 3.5) lost much of its negative charge, promoting weaker electrostatic bonds and solubilizing the complexes formed previously. Similar pH transitions in the formation of complexes between BSA and Sugar Beet Pectin (SBP) were described by Li et al. (2012).

Previous studies have shown that the turbidity of an isolated PEC solution remains close to zero over the entire pH range (7.0 to 1.5) indicating that PEC does not form aggregates that can disperse light and interfere with turbidimetric analysis (Jones et al., 2009). Likewise, it has been demonstrated in the literature that BSA does not form aggregates at this pH range (Santos et al., 2018). Thus, it could be concluded that the observed turbidity was directly related to the formation of the complexes.

When analyzing Figure 1a, different ratios resulted in displacements of $pH_{Ø1}$. At the lowest ratio studied (1:1), we observed a very marked displacement at $pH_{Ø1}$, reducing the pH_{max} range. In the ratios above 1:1 a wide range of formation was verified with the highest formation range observed in the ratio (10:1), due to the displacement of $pH_{Ø1}$. This dependence of $pH_{Ø1}$ for different reasons was verified by Muhoza et al. (2019) with gelatin and High-Methoxylated Pectin (HMP) where minor reasons shifted the formation of complexes to a lower pH.

In the higher ratios (5:1 and 10:1), the interaction force between the protein and the polysaccharide was strong enough to virtually suppress the formation of soluble complexes (pH_c), resulting in rapid increase in turbidity and insoluble complex formation ($pH_{Ø1}$). By means of the turbidity variation analyzed in Figures 1a, it could be concluded that the turbidity was larger and more comprehensive in the 10:1 ratio of BSA:PEC. According to the literature, the best formation ratio is related to the load balancing of the biopolymers. Li et al. (2012) found that the best ratio between BSA and SBP was 8:1. Ru et al. (2012) studied the rheological behavior of complexes between BSA:PEC and found that maximum shear modulus is observed in the 10:1 ratio. According to the authors this could suggest that the density of the positive charge on BSA was approximately equal to that of the negative charges on PEC, and their interaction was almost at a maximum. In contrast, Muhoza et al. (2019) found the 3:1 ratio for complex coacervates formed between a high molecular weight fibrous protein, gelatin, and HMP, reinforcing that the biopolymer ratio for coacervation is achieved from load balancing.

For the BSA:GA systems (Figure 1b), a first region between pH 7.0 and 5.5 ($pH > pH_c$) was found where the polymers were in the soluble form, thus verified by the turbidity close to zero (Lv et al., 2014; Aryee & Nickerson, 2012). In fact, during titration and pH reduction, the protein begins to assume a positive charge, and near pH 5.0 a gradual increase of the turbidity is observed, this pH being denominated as pH_c. From the pH 4.5 near the isoelectric point of the protein there was an abrupt increase of the turbidity ($pH_{Ø1}$) and around the pH 3.5 the turbidity presented its maximum value. At pH 2.5 low turbidity values indicated the start of the dissociation of the biopolymers and at pH 2.0 the end of the complexation ($pH_{Ø2}$) because GA had few negative charges. As observed in the results, the BSA: GA complexes showed a maximum pH for each ratio and not a maximum pH range as verified for the BSA: PEC complexes. This behavior limits its application to specific pH conditions.

Previous studies by Aryee & Nickerson (2012) have shown that the turbidity of an isolated GA solution remains close to zero over the entire pH range (7.0 to 1.5) indicating that GA does not form aggregates that can disperse the light. The occurrence of complexation was previously reported for pea proteins (PPI) and GA (Liu et al., 2009) and ovalbumin and GA (Girard et al., 2003) and a peak near pH 3.5 was found.

As for PEC, the ratio of protein: polysaccharide (r) mass had a great impact on the formation of complexes between BSA and GA. In the ratio 1:1 the values of pH_c and $pH_{Ø1}$ moved considerably suggesting that the interactions were significantly reduced due to the low protein content. The ratios of 3:1 to 10:1 showed similar values of pH_c and $pH_{Ø1}$. These results could suggest that the best characteristic associated with the interaction between BSA and GA was that of 3:1, because it is assumed that in this ratio the polysaccharide

became saturated with protein chains and in larger ratios the proteins were in excess, not influencing the formation of coacervate complexes (Liu et al., 2009; Aryee & Nickerson, 2012). The reasons found by Niu et al. (2014) for complexes formed between Ovalbumin and GA and by Liu et al. (2009) for PPI and GA was 2:1 and 1:1, respectively.

In a turbidimetric study and by dynamic light scattering, Vinayahan et al. (2010) verified the formation of insoluble complexes in ratio 2 at pH 4.7. Different results may be related to the use of slow acidification achieved by GDL. In the same study, the analysis regarding Isothermal Titration Calorimetry (ITC), revealed that the complexation between pH 3.0 and 4.0 would consist of 60 molecules of BSA per GA molecule, whereas at pH 5.0 of 10 to 1.

Figure 2 illustrates solutions containing BSA:PEC (10:1) (Figure 2a) and BSA:GA (3:1) (Figure 2b) and have order to evaluate the variation of turbidity to fixed pH and ionic strength, at different pH along the turbidimetric titration. For BSA:PEC solution, we found a totally cloudy solution from pH 3.8 to 2.7 and from pH 4.2 to 3.4 for BSA:GA similar to the results shown in Figure 1.

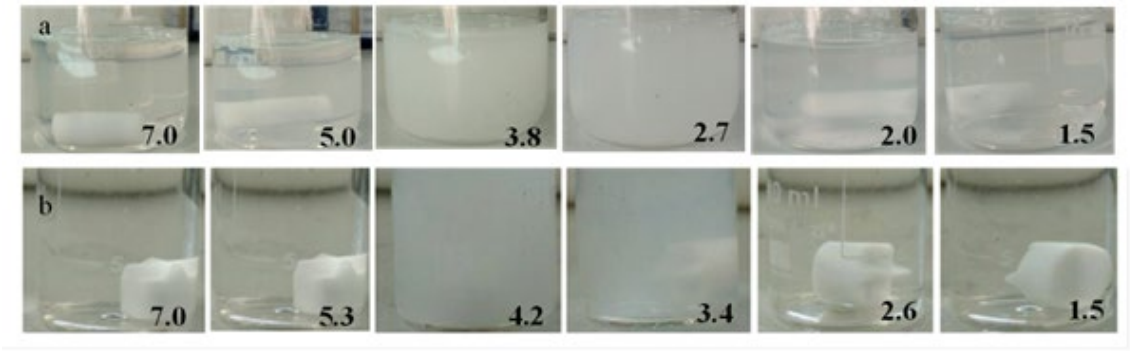

Figure 2. (a) Photographs of solutions containing BSA:PEC (10:1) and (b) BSA:GA (3:1) at different pH.

3.2 Potencial- ζ

The potential-ζ (mV) as a function of pH (2.0-7.0) was performed to evaluate the charge density of the isolated polymers (BSA, PEC and GA) and the complexes formed at different ratios (r) and the results are shown in Figure 3. According to Figure 3a, BSA showed a positive potential of +26.4 at pH 2.0 and as pH increased, the potential decreased, reaching zero load at pH 4.7, this being its isoelectric point, as reported in the literature, and decreased to -21.9 mV potential at pH 7.0. Similar results were found by Santos et al. (2018). In Figure 3a, with respect to PEC as an anionic polysaccharide, it could be showed a negative charge of pH 7.0 at pH 2.0 reaching -42.1 mV potential at pH 7.0 and potential-ζ of -2.1 mV at pH 2.0, as observed by Muhoza et al. (2019). As regards Figure 3b, GA presented a negative charge throughout the studied pH range reaching a potential-ζ of -35.1 mV at pH 7.0 and -2.7 mV at pH 2.0 similar to that reported by Vinayahan et al. (2010)

In general, the ratio of protein:polysaccharide (r) has a great impact on the degree of complexation. In low ratios (high concentration of polysaccharides), the complexes are highly negative because there are few protein molecules linked to the anionic polysaccharide. As the ratio increases (the polysaccharide concentration decreases), the charge on the complexes becomes less negative due to the higher amount of proteins. Thus the protein becomes available to neutralize the negative charges of the carboxylic groups even when pH > pI, (Girard et al., 2003) which gradually increases the strength of the interaction between protein and polysaccharide resulting in displacement $pH_{Ø1}$ and pH_c.

Complexes containing different ratios of BSA:PEC (Figure 3a) and BSA:GA (Figure 3b) showed intermediate zeta potentials and pI's varying from 2.6 to 4.6 and 3.7 to 4.2, respectively, indicating that the BSA was strongly bound to the polysaccharides and meant that the interaction occurred when the charges between the polymers were opposite, probably through electrostatic interactions between the anionic carboxyl groups of the polysaccharide and the cationic amino groups of BSA (Jones & Mc Clements, 2010). As the ratio increases, the pI's of the complexes move in a positive direction and the instability increases. It is possible to verify that approximate values of pI of the mixtures (BSA:PEC and BSA:GA) coincide with the maximum turbidity point observed in Figure 1a indicating that the interaction occurred more intensely when there was load neutrality of the biopolymers (Liu et al., 2015).

The study of coacervate complexes through the analysis of zeta potential had the purpose of identifying the ideal pH for each ratio (protein: polysaccharide) studied. From these results, it was possible to choose the ratio and the most suitable pH for the specific conditions of a given product in which it is intended to apply these complexes.

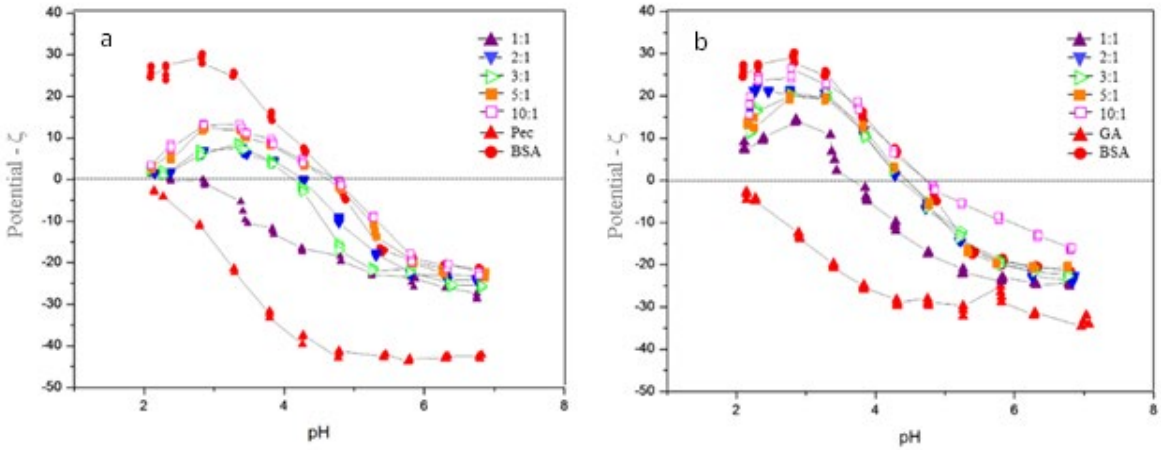

Figure 3. pH-dependent potential of the samples containing (a) PEC and BSA:PEC and (b) GA complexes and BSA:GA complexes in different ratios.

3.3 Effect of ionic strength on the formation of coacervated complexes

Figure 4 shows the influence of NaCl on the formation of coacervate complexes between BSA:PEC (10:1) (Figure 4a) and BSA:GA (3:1) (Figure 4b) by turbidimetric analysis at different concentrations of NaCl. When analyzing Figure 4a concerning the complexes between BSA:PEC, we could see that the turbidity of all curves were practically constant from pH 7.0 to 5.0 and had a low turbidity value until reaching $pH_{Ø1}$. After this value the turbidity could increase and reach maximum values at all different concentrations of NaCl. However, the turbidity of the systems behaves differently at the salt concentrations studied when pH decreases from 5.0 to 2.0. At the concentrations of NaCl ≤ 0.10 mol L^{-1}, a shift from $pH_{Ø1}$ to lower values was observed, whereas $pH_{Ø2}$ showed no dependence on the NaCl concentration, as it did not change. At 0.40 mol L^{-1} there is no noticeable increase in turbidity. This is because at this concentration the competitive adsorption capacity between the Na^+ and Cl^- ions becomes sufficiently strong to suppress the electrostatic interaction between the biopolymers, which explains the less turbidity of the system. These results are in agreement with those found by Li et al. (2012) which at concentrations of NaCl ≤ 0.2 mol L^{-1} they did not observe reduction in turbidity for BSA and SBP. According to works by Ru et al. (2012) the increase of the salt concentration reduced the range of formation of the complexes, however, it was not verified turbidity reduction in the concentration of 0.40 mol L^{-1} to 5:1 ratio.

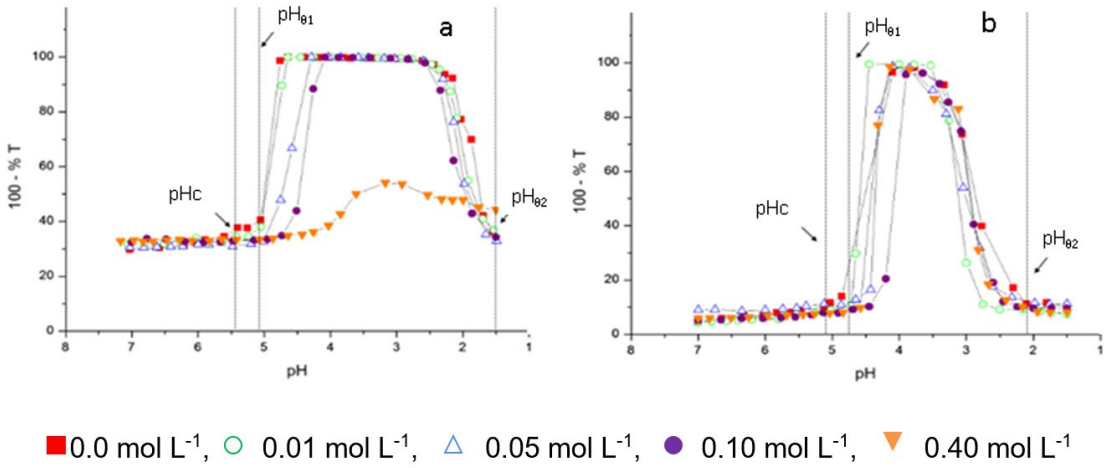

Figure 4. Influence of different NaCl concentrations on the turbidity of samples containing (a) BSA:PEC in a ratio of 10:1 and (b) BSA:GA in a ratio of 3:1.

Contrary to what was observed for the complexes between BSA:PEC, according to Figure 4b, the formation of complexes between 3:1 BSA:GA ratio was not reduced at the salt concentrations studied. Different results were verified by some authors between GA and different proteins confirming that the sensitivity of the complex to the salt may vary in function of the studied biopolymers, being able to be in conflict with the intermolecular interactions or still to be related to other parameters of the analysis as the type of salt, total concentration and biopolymer ratio (Vinayahan et al., 2010; Weinbreck et al., 2003; Niu et al., 2014).

It can be further noted that when increasing the NaCl concentration, the $pH_{Ø1}$ to $pH_{Ø2}$ range for the BSA:PEC coacervates (Figure 4a) and BSA:GA (Figure 4b) were reduced and this result was also observed by Weinbreck et al. (2003). The change in $pH_{Ø1}$ dependent on ionic strength is a known phenomenon. In general, the presence of salt in solution weakens the interaction, and may even suppress the formation of coacervate complexes between protein and polysaccharide (Weinbreck et al., 2003). We can attribute the effect caused by NaCl to the fact that the protein molecules are amphoteric polyelectrolytes, i.e., they are characterized as polymers containing both positive and negative charges, so there are attractive and repulsive electrostatic forces between the charges of proteins and polysaccharides (Hattori et al., 2000). The complexation is driven by electrostatic interactions but is influenced by the entropy of the system which increases due to the expulsion of small ions from the double layers around the chains of individual polyelectrolytes (Biesheuvel & Stuart, 2004). A large amount of salt hinders the release of these ions, effectively reducing the available active sites for the interaction between the polymers and suppressing the complexation (Espinosa-Andrews et al., 2007). The variation in the salt concentration limit for coacervation may be related to the different natures of the polymers, including differences in charge density due to branching and different pKa values (Priftis et al., 2014).

3.4 Fourier Transform Infrared spectroscopy (FTIR spectroscopy)

The FTIR spectra of BSA, PEC and BSA:PEC are shown in Figure 5a and BSA:GA in the Figure 5b. According to the literature, the most sensitive regions of FTIR spectra associated with the structure of BSA refer to amides I, II and III. The major FTIR spectra observed for BSA (Figure 5) are between the bands 1300 cm^{-1} to 1700 cm^{-1}. The peak observed at 1640 cm^{-1} represents the amide I being formed by stretching the C = O (free carbonyl) group, the 1526 cm^{-1} peak for the drawing of the NH groups represents the amide II, and the amide III refers to the 1393 cm^{-1} peak corresponding to the stretching of the CN and NH groups (Stuart, 2006; Huang et al.,

2006). In addition to the amides, the stretching of the N-H and O-H groups near 3300 cm^{-1} (Barth & Zscherp, 2002) was verified. A similar BSA profile was also found by Santos et al. (2018).

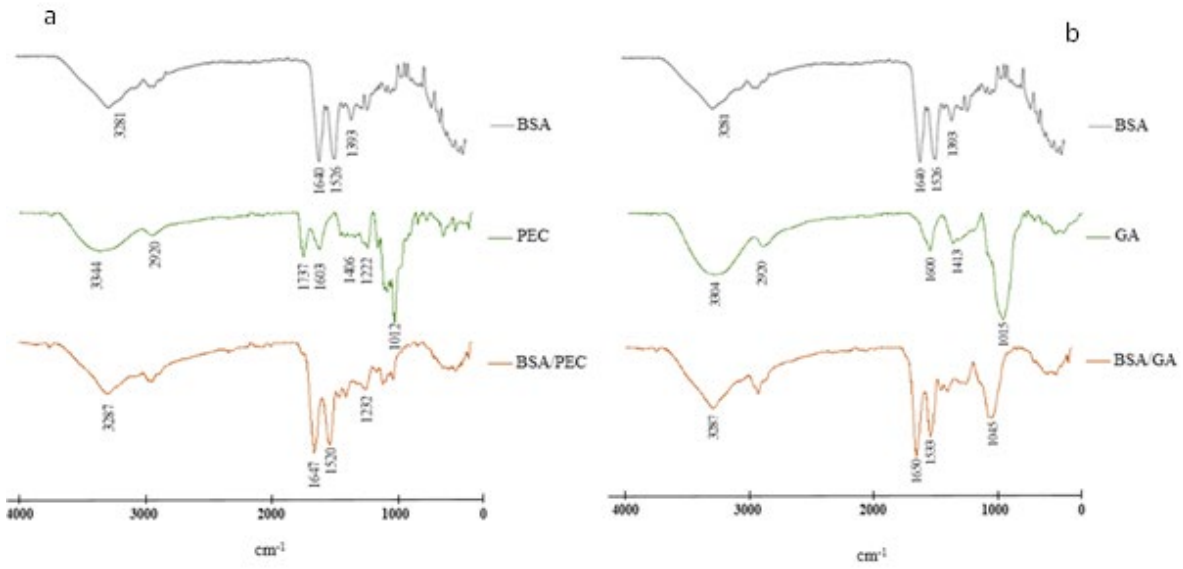

Figure 5. FTIR spectra for (a) BSA, PEC and BSA:PEC (10:1) at pH 4.5 without addition of NaCl and (b) BSA, GA and BSA:GA (3:1) at pH 4.0 without addition of NaCl.

The FTIR spectrum of PEC depends on the origin of the polysaccharide and the degree of methylation of carboxyl groups. By means of the FTIR spectra presented (Figure 5a), it was possible to identify the axial deformation of O-H, observed in the intense and very wide band in the region of 3.600 to 3.000 cm^{-1}. The band in the region of 2920 cm^{-1} is attributed to the vibration of the C-H bonds. The band in the region of 1737 cm^{-1} corresponds to the axial deformation of the conjugated ester (C = O in ester group) and the band at 1603 cm^{-1} is attributed to the asymmetric deformation of the COO- group. Similar results were also observed by Slavutsky & Bertuzzi (2019). The carbohydrates have strong absorption between 1200 and 950 cm^{-1}, and this region is associated with the characteristic fingerprint for each polysaccharide. According to Gnanasambandam & Proctor (2000) these bands are often difficult to interpret. For PEC, this region was identified at the peak at 1012 cm^{-1}, equal to that found by Lan et al. (2020)

As for PEC, a broad and intense band in the region of 3600-3000 cm^{-1} was observed for the GA sample (Figure 5b) due to the stretching of the -OH- groups and a 2920 cm^{-1} peak characteristic of carboxyl groups (negatively charged), is due to the stretching of the CH group. In 1600 cm^{-1} the polymers also showed the characteristic band of C = C stretch. The band at 1413 cm^{-1} represents C = O symmetric stretching, being in agreement with those found by Daoub et al. (2016). The fingerprint region in GA was located at the peak at 1015 cm^{-1}

According to the results, we found that there were differences between the spectra of the individual biopolymer and the spectra of the complexes, in this sense, we could conclude that there were intermolecular interactions between the carboxyl groups in the structure of the polysaccharides and the functional groups of the protein. When comparing the spectrum of complex coacervates for BSA:PEC (Figure 5a) and BSA:GA (Figure 5b) with the BSA spectrum, we verified a displacement and an increase in the intensity of the bands referring to the amides. The increase in these bands would be related to the occurrence of electrostatic interaction between the amide groups of BSA (NH_4 +) and the carboxyl groups of GA and PEC (COO-). Similar results were found by Raei et al. (2018) for WPI and PEC complexes.

In addition, the spectrum of the complexes showed a broad band around 3000-3600 cm^{-1}, indicating an increase of hydrogen bonding. This implies that the hydrogen bonding was also involved in the interaction

between the biopolymers. The complexes showed a peak in the fingerprint region that could be an overlap of the protein and polysaccharide. In BSA/GA complexes, this peak was much more intense than in BSA/PEC. These results may be associated with the protein: polysaccharide ratio considering that the presence of the polysaccharide in BSA/GA (3:1) was greater than in BSA/PEC (10:1)

The spectra of the complexes reflect the formation of a structure that could exhibit specific spectra of protein as well as of polysaccharides, confirmed the interaction between them through coacervation, but also suggested the type of interactions involved in that process that included hydrogen bonds and electrostatic interactions.

4 Conclusions

This study demonstrated that the interaction between BSA and different polysaccharides (PEC and GA) may result in the formation of soluble and insoluble complexes as a function of pH. The presence of NaCl negatively influenced the formation of the complexes. BSA: PEC complexes were more sensitive to high salt concentrations than BSA: GA. Their formation was also influenced by the ratio having been intensified as a function of the protein increase in the system (BSA:PEC 10:1; BSA:GA 3:1). The PEC exhibited a higher charge density compared to GA which required more positive BSA charge for neutralization and complexation. Through the turbidimetric analysis, it was possible to identify which BSA: GA complexes were formed under specific conditions of pH (3.5) while BSA: PEC complexes exhibited a behavior that allows their application in different food products (4.9 to 1.5). Observed changes in the spectra (FTIR) of the complexes indicated the participation of hydrogen and electrostatic bonds in their formation. The interaction between proteins and polysaccharides through complex coacervation constitutes a technological alternative, considered a clean technology that does not cause an increase of polluting agents to the environment. The coacervates are attractive to the industry because they are natural products obtained from inexpensive and easily approved ingredients for use in food. Being formed a biopolymer differentiated functional property able to microencapsulate bioactive ingredients.

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