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Production of microparticles with gelatin and chitosan

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ABSTRACT

In the past few decades, the textile industry has significantly increased investment in research to develop functional fabrics, with a special focus on those aggregating values. Such fabrics can exploit microparticles inferior to 100 μm, such as those made by complex coacervation in their creation. The antimicrobial properties of chitosan can be attributed to these microparticles. Developing particles with uniform structure and properties would facilitate the control for the eventual release of the core material. Thus, a complex coacervation between gelatin and chitosan was studied, and the optimal conditions were replicated in the encapsulation of limonene. Spherical particles formed had an average diameter ($D_{3,2}$) of 30 μm and were prepared with 89.7% efficiency. Cross-linking of these microparticles using glutaraldehyde and tripolyphosphate was carried out before spray drying. After drying, microparticles cross-linked with glutaraldehyde were oxidized and clustered and those that were cross-linked with tripolyphosphate resisted drying and presented a high yield.

1. Introduction

Since the installation of the first loom in 1874, the textile industry has played a central role in the development of the Brazilian economy. Brazil is the fifth largest producer of textiles in the world (IEMI-Instituto de Estudos e Marketing Industrial, 2012) with jobs in the textile chain currently representing 16.4% of the jobs in the country. In the manufacturing industry, textiles is the second largest employer, second only to the combined food and beverage industry (ABDI, 2010). However, despite its impact on the economy, this industry has historically invested in innovative technologies only in response to crises. The Industrial Revolution gave rise to the mechanization of large-scale production, which is still important today for competing with products from foreign markets, especially those from Asia, which are sold on the Brazilian market at extremely low prices.

Some countries such as Germany and Portugal are managing to overcome Asian competition by investing in the development of technologically advanced products, such as functional and intelligent textiles. Such fabrics exhibit special properties, such as waterproofing, anti-flammability, conductivity, memory for shape, insect repellency, and various reactions to light and temperature. Their development is possible due to the combination of different technologies. In this context, microparticles emerge as a strong contender for innovation, and their use has become a major challenge for researchers. Microparticles have established new frontiers for textile applications because they can possibly convert ordinary products into articles of high performance, with a strong technological component making them able to respond to the most adverse situations, such as those found in medical, high-level sports, and military settings (Soutinho, 2006).

These particles can be applied at any stage during the processing of fibers, making them functional and imparted with durable color or fragrance (Feczkó, Varga, Kovács, Vidóczy, & Voncina, 2011; Monllor, Bonet, & Cases, 2007; Rodrigues et al., 2009; Sawada & Urakawa, 2005), cosmetic properties (Alonso, Gimeno, Sepúlveda-Sánchez, & Shirai, 2010; Lam et al., 2012; Nelson, 2002; Wang, Shi, Cheung, & Xin, 2011), the ability to repel insects (Specos et al., 2010) and properties protecting against microbes and promoting healing (Abramov et al., 2009; El-Rafie, Mohamed, Shaheen, & Hebeish, 2010; Saraswathi, Krishnan, & Dilip, 2010; Yang et al., 2012), etc. Textile fibers can also be modified to present thermal properties (Ribeiro, Silva, & Soares, 2013; Salaün, Devaux, Bourbigot, & Rumeau, 2010; Sánchez, Sánchez-Fernandez, Romero, Rodríguez, & Sánchez-Silva, 2010; Shin, Son, & Yoo, 2010).

Microparticles can be used for various purposes, but the most common are protection and control of the release of the core

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material. They can be produced by chemical or physical processes and correspond to small structures containing droplets of an active compound dispersed or surrounded by a wall material. Several techniques have been used for the development of microparticles for textiles (Alonso et al., 2010; El-Rafie et al., 2010; Hsieh, Chang, & Gao, 2006; Mihailović et al., 2010; Rodrigues et al., 2009; Specos et al., 2010), but complex coacervation is leveraged due to the typically high encapsulation efficiency and the interaction between two polymers to form a shell surrounding the oily core material. Core materials provide functionality to the fiber, but further benefits can develop from the use of adequate wall material, such as chitosan, which favors antimicrobial activity.

Coacervated microparticles are obtained in a wet form but can be dried to expand the possibilities for incorporation into textiles, requiring the strengthening of the polymer matrix to increase the resistance of the microparticles to thermal processes. However, complex coacervation does not guarantee the formation of isolated microparticles and can sometimes result in a matrix with a widely dispersed active component. Spherical and isolated microparticles are, however, preferable for providing better control of the release of the core material, when necessary, as well as for guaranteeing homogeneity in the distribution of the active compound throughout the fabric. The objective of the present paper was to determine the conditions for the formation of chitosan-gelatin coacervates, describing their characteristics and evaluating the use of different cross-linking agents in the stabilization of these particles during drying.

2. Experimental

2.1. Materials

The materials used as wall material included Type-A gelatin (Tovani Benzaquen Rep. Ltda, Gelita CW100, LFP722611, São Paulo, SP, Brazil), Type-B gelatin, from bovine skin (gel strength 244 bloom, LF 21502/04, Gelita South America, São Paulo, SP, Brazil), chitosan (Polymar Ciência e Nutrição, 20100210, DA = 89%, MW = 69.000 g mol^{-1}, Fortaleza, CE, Brazil) and gum Arabic (IRX49345, supplied by Colloides Naturels Brasil Comercial Ltda, São Paulo, SP, Brazil). Core material included limonene oil (95% pure, orange essential oil, Citrosuco S/A, Bebedouro, Brazil). All other reagents were of analytical grade, and deionized water was used in all experiments.

2.2. Methodology

2.2.1. Evaluation of free surface charge

Gelatin solutions (1% and 3%, w/v) were prepared by dissolving the protein in distilled water and stirring for 1 h at 50 °C until complete hydration. Chitosan solutions (1% and 3%, w/v) were prepared by dissolving equivalent masses in acetic acid solution (1%, v/v) and stirring for a minimum of 12 h at room temperature and filtered through 1 μm pore size filters.

Static zeta potential (mV) was measured with a Zetasizer 2000 (Malvern Instruments Ltd., Worcestershire, UK). The superficial load of diluted polymeric solutions (0.5%, w/w) was determined for a range of pH from 4.0 to 9.0 using automatic titration with HCl (0.5 N) or NaOH (0.5 N). These solutions were maintained under agitation at 50 °C, with rapid reading used to prevent the cooling and consequent gelation of the protein.

2.2.2. Visual evaluation of polymeric pair behavior

The stock solutions (1% and 3%, w/v) were mixed at ratios pre-established on the basis of the zeta potential analysis (30:1, 20:1, 10:1 and 1:1 gelatin:chitosan). The absorbance of these mixtures was determined by spectrophotometric measurement (Specord UV VIS, Carl Zeiss, Jena, Germany) at λ = 590 nm for a pH ranging from 3.0 to 6.0 (50 °C). All measurements were performed after equilibrating the coexisting phases for 15 min after pH adjustment. Concentrations greater than 3% (w/v) were not studied due the limited solubility of the chitosan. For all combinations of pH and ratio, visual aspects were captured using a camera (Eclipse T800 microscope, Nikon, Japan), and the zeta potential was measured as described above.

2.2.3. Experimental confirmation of microparticle production

An estimate ratio of the polymers was defined by turbidimetry and zeta potential analysis was used to adapt the traditional protocol for particle production with gum arabic and gelatin (Prata, Zanin, Ré, & Grosso, 2008). Problems with the polymers complexation were found, and certain strategies had to be adopted to be able to produce spherical particles. One of these includes the eventual addition of a solution of sodium sulfate (1%, w/v). This addition was attempted prior to homogenization, either to the gelatin or to the mixture of the polymers. These experimental procedures and results are described briefly in the results section.

2.2.4. Microparticles preparation

The optimal conditions for the production of essential oil-loaded microparticles were used, as follows: an emulsion with 100 mL gelatin (1%, w/v) and 0.55 g of limonene essential oil at 50 °C was prepared through homogenization at 14,000 rpm in a T-18 homogenizer (IKA Works, Inc., USA). This emulsion was then added to 10 ml of chitosan solution (1%, w/v), pre-adjusted to a pH of 6.0 also at 50 °C. A rapid adjustment of the pH of the mixture was made to the pH of 6.0, and the system was slowly cooled for 3 h to a temperature of 10 °C.

2.2.5. Microparticles yield and encapsulation efficiency

After coacervation, the produced microparticles were stored in a refrigerator at 4 °C for 16 h. The precipitated microparticles were separated using a sieve (ASTM 170 – mesh 0.090 mm) and weighed. The moisture content of these microparticles was determined using a vacuum oven technique for 24 h, at 80 °C, in triplicate. The yield of the process was calculated as the percent of dry material precipitated (minus the content of oil determined) in relation to the initial dry mass (mass of polymers on a dry basis).

Determination of the content of essential oil-loaded in microparticles was made by spectrophotometry. Ethanol (10 mL) was added to dried and crushed microparticles (0.5 g) in a tube with a lid. The system was vigorously agitated for 15 min, and agitation of the tube was maintained over the subsequent 8 h. The supernatant was collected after 24 h with a Pasteur pipette with a piece of cotton on the tip to function as a filter for the microparticles. Absorbance was measured at a wavelength of 320 nm using UV–vis spectrophotometer (Specord UV VIS, Carl Zeiss, Jena, Germany). The amount of essential oil in the microparticles was calculated by appropriate calibration curve of free d-limonene in ethanol (Abs = 11.55 [Conc] + 0.0033; R^2 = 0.992). Each batch of samples was measured in triplicate. The encapsulation efficiency (EE) was calculated as:

EE = mass of encapsulated oil/mass of oil in the process

2.2.6. Cross-linking of microparticles

Cross-linking was performed with either glutaraldehyde (GLU) or sodium tripolyphosphate (TPP). Both were added drop wise, at room temperature, to a solution of microparticles under constant stirring for 3 h. GLU was used at a concentration of 1 mmol g^{-1} of polymer and TPP solution (3%, w/w) was added to the wet particles to obtain 0.068 g of TPP g^{-1} of chitosan. After cross-linking, the

particles were then washed with distilled water and filtered through a 10 μm sieve.

2.2.7. Spray-drying of microparticles

Spray drying was performed in a laboratory scale LabPlant SD-06 spray-dryer (Huddersfield, England), with a 1.5 mm diameter nozzle and main spray chamber of 500 mm × 215 mm. The emulsion was fed into the main chamber through a peristaltic pump at a feed flow rate of 8 g min^{-1}, with a drying air flow rate of 73 m^3 h^{-1} and a compressor air pressure of 0.06 MPa. Inlet temperature was 140 °C. The assay was performed in triplicate.

2.2.8. Microparticle size and morphology

Moist microparticle size was determined using laser light scattering (Hydro 2000 MU, Malvern Mastersizer, United Kingdom). Triplicate samples were analyzed and mean volumetric diameter ($D_{4,3}$) was reported. Morphology was observed using an optical microscope (Eclipse E800, Tokyo, Japan), coupled with a digital camera, and registered using Image Pro Plus 4.0 software.

The morphology of the spray-dried microparticles was evaluated by scanning electron microscopy (SEM) (Hitachi Tabletop Microscope, Hitachi HTA Inc., USA) with 15 kV.

3. Results

3.1. Analysis of polymers complexation

One methodology widely used in the selection of optimal processing conditions for microencapsulation by complex coacervation is the determination of available charges of the polymers. This can be estimated by zeta potential measurement, which can significantly reduce the number of assays required for complex formation because the range of probability for interaction can be determined.

Fig. 1 shows the distribution of charges for chitosan as well as the two types of gelatin: that obtained by acid hydrolysis (type A) and the other by alkaline hydrolysis (type B).

Polysaccharides belong to the carbohydrates family with a structure consisting of rings of sugars linked by easily hydrolyzable (glycosidic) links. The relation between the charge and the pH of carbohydrates depends on the structure, but is generally due to the degree of dissociation (pKa) of the carboxylic groups ($COOH \leftrightarrow COO^- + H^+$) when the pKa is approximately 3.5, or ionization of the amino groups ($NH_2 + H^+ \leftrightarrow NH_3^+$), when the pKa is between 7.0 and 10.0 (Crouzier, Boudou, & Picart, 2010), such as in the case of chitosan. A similar mechanism is also relevant for proteins, with the isoelectric point (IP) indicating the neutral charge point.

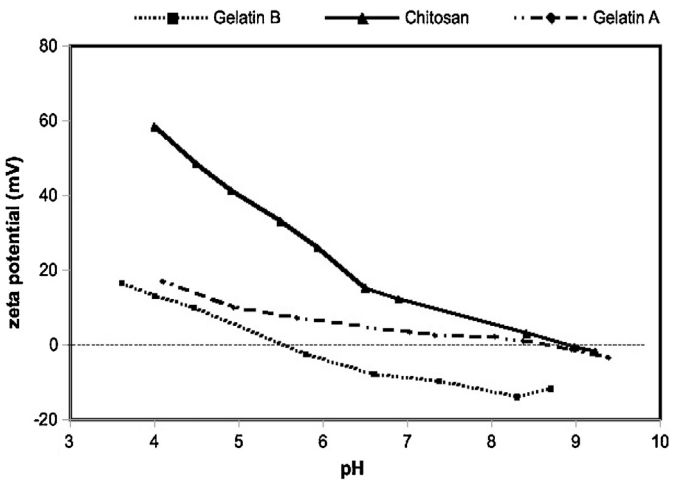

Fig. 1. Effect of pH on zeta potential of colloidal species in solution. Null charges of Type A gelatin (8.9), Type B gelatin (5.5) and chitosan (8.9).

Type A gelatin has a positive charge for a wide range of pHs because its IP is approximately 8.9, indicating an excess of positively charged amine groups. For Type B gelatin, an increase in pH leads to the dissociation of the carboxylic groups, resulting in a very low IP and the development of an excess of negative charges for a pH above 5.5, reaching a minimum of −15 mV at pH 8.0.

The amino group of chitosan has a pKa of 6.5, and this polymer presented an excess of positive charge for the full range of pH values studied. The development of positive charges decreased strongly with an increase in pH, from 55 mV at pH 4.0 to 10 mV at pH 7.0. Chitosan is thus presented in the cationic form and can react with negatively charged molecules.

An analysis of Fig. 1 rules out the use of type A gelatin for complexation with chitosan because it has no regions of opposite charges. The complexation of chitosan with type B gelatin, however, is possible, at least theoretically, for a pH between 5.5 and 8.5.

The establishment of this range is the first guideline for the obtention of microparticles with this pair of polymers. However, the solubility of chitosan depends on pH, and thus the insoluble form should be not used for interactions with another polymer. Visual aspects of diluted solutions of chitosan (1% and 3%, w/v) in a pH ranging from 3.0 to 9.0 were followed, and the increase in pH caused the development of cloudiness and the gelation of the system. Because the solubility of chitosan is related to the amount of protonated amine groups ($-NH_3^+$) in the polymer chain, to solubilize chitosan the pH must be less than its pKa in solution. This is because, above this value (pKa 6.5), more than 50% of amino groups are deprotonated and become insoluble. Thus, the range of pH estimated by the density of the surface charge for the polymers was restricted to between 5.5 and 6.5, considering the insolubility of chitosan.

3.2. Establishment of an ideal ratio for complex coacervation

The next step for particle formation is the optimization of production conditions such as the ratio between polyelectrolyte and the polymer concentrations, as well as temperature and rate of pH reduction. The ratio of the two biopolymers in complexation was determined by the analysis of the curve of the zeta potential for the individual solutions.

Take for example, the line passing through the 6.0 pH value in Fig. 1. Type B gelatin has a charge of about −3 mV, and chitosan of approximately 28 mV. It would thus be necessary to use 9 times as much gelatin as chitosan for its negative charges to neutralize the charges exhibited by the chitosan. Thus, a ratio of the polymers should be set at 9:1. Considering that the neutralization of charges at pH 5.5, 6.0 and 6.5 was 30:1, 9:1 and 2:1, respectively, the proportions of 30:1, 20:1, 10:1 and 1:1 for gelatin–chitosan were used, respectively, for deeper studies.

Turbidity measurements were performed at a wavelength of 500 nm for the mixtures of chitosan and gelatin and the influence of the two total polymer concentrations (1% and 3%, w/v) could be evaluated. The results are shown in Figs. 2–4. Together with these data the visual appearance is shown of the solutions and the value of charge density (mV).

Fig. 2A and B, which correspond to ratios of 1:1 and 10:1, respectively, clearly show an excess of chitosan in the system, with the development of positive charges even with high complexation of the polymers. For both ratios, a discrepancy between the visual appearance (absorbance) and the behavior of free charges was observed. At a ratio of 10:1, the maximum turbidity was observed at pH 6.0, and the zeta potential value reduced continuously up

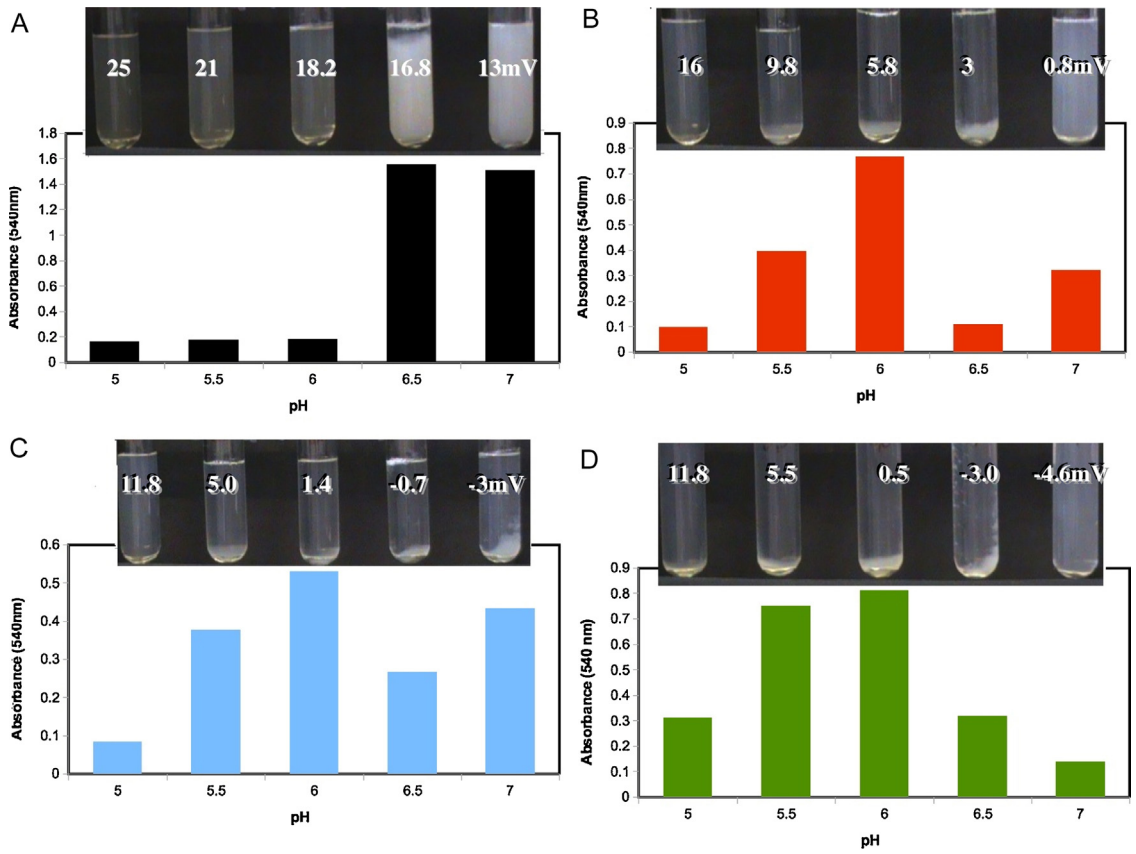

Fig. 2. Solution at 1% (w/w) with various ratios of gelatin to chitosan. (A) 1:1; (B) 10:1; (C) 20:1; (D) 30:1.

to pH 7.0 yet maintained an excess of positive charges (+0.8 mV), indicating that the ideal ratio for complexation between polymers has not been reached. The reduction of the free charges at pH 7.0 was influenced by the alkali addition, which tends to neutralize the negatively charged group of the chitosan.

For ratios of 30:1 and 20:1 (Fig. 2C and D) an increase in the absorbance of the mixtures of polymers up to pH 6.0 was consistent with those estimated by the charge density, but with this last, the optimum pH was more accurate: pH 5.5 for the ratio 30:1 and pH 5.75 for 20:1. An excess of negative charges was observed for pH values greater than 6.0, indicating that all the available charges of the chitosan had been complexed, which suggests that a pH of 6.0 should be maintained for complex coacervation with these proportions.

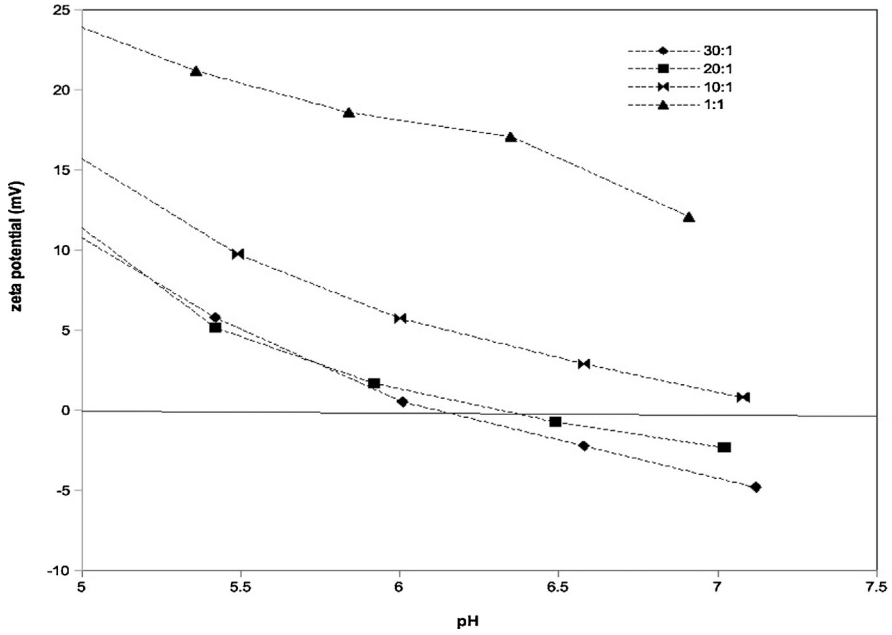

Fig. 3. Zeta potential of the mixtures containing different ratios of gelatin:chitosan.

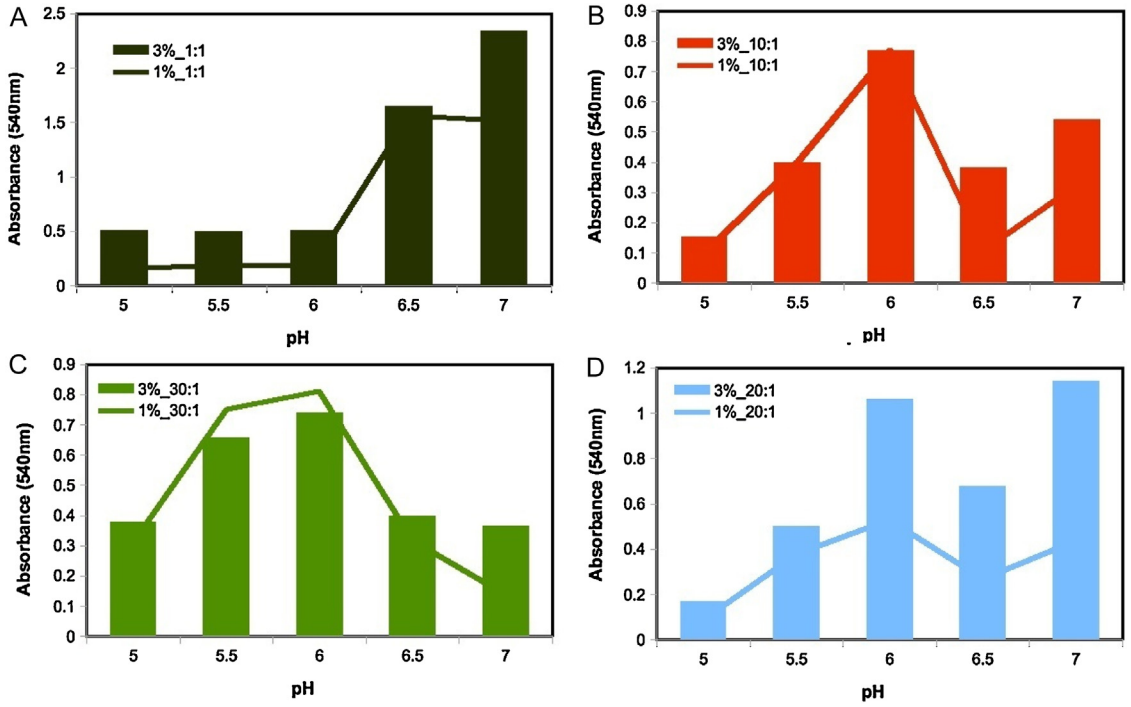

Fig. 4. Influence of concentration of polymer in the absorbance of the mixtures containing gelatin:chitosan. (A)1:1; (B) 10:1; (C) 30:1; (D) 20:1.

The charge distribution patterns developed by the mixtures of polymers can best be seen in Fig. 3. In mixtures of 20:1 and 30:1 gelatin:chitosan, the equivalency of charges was reached at a pH of approximately 6.0. Remunan-Lopez and Bodmeier (1996) evaluated the formation of coacervates for a ratio of 20:1 gelatin:chitosan, and the highest yield was found between pH 5.25 and 5.5. Moreover, Kang, Dai, and Kim (2012) found that at pH 6.0 the highest transmittance was obtained for a ratio of 15:1. These values are very close to those established here.

Similar behavior to that observed for a ratio of 10:1 was found for 1:1. However, in this ratio turbidity did not reveal the formation of complexes for any pH below 6.0, and high net charges (20 mV) characterized this mixture. The measurement of zeta potential is a relative indicator of colloidal stability. High zeta potential values for a mixture, whether positive or negative, indicate the repellence of the polymeric chains, thus inhibiting aggregation. Low absolute values, however, indicate agglomeration, and the dispersion becomes unstable and precipitates (Morrison & Ross, 2002).

For the most complex systems, turbidity represents the formation of insoluble complexes. However, as chitosan has a large amount of reactive groups, the relative proportion of amino and amide groups in the macromolecular chains changes with pH, and thus pH has a marked effect on solubility. Turbidity of systems with a pH greater than 6.5 thus represents insolubility of the chitosan rather than the formation of complexes. For all ratios at pH 7.0, all systems remained cloudy, even those with a negative zeta potential, as in the case of the ratios of 20:1 and 30:1.

The relationship between insolubility and increase of absorbance is extremely important in justifying the use of complementary techniques for the study of complex coacervation. In some cases (as mentioned in Prata (2006) for gelatin – gum Arabic) spectrophotometric measurements faithfully correspond to the results obtained with the zeta potential and could be used exclusively.

Turbidity can also be used to assess the intensity of complexation once greater absorbance values correspond to greater complex formation. Comparing the ratios 20:1 and 30:1, the absorbance for these mixtures increased from 0.53 to approximately 0.8, respectively, indicating increased production of coacervates when a higher proportion of gelatin was used.

Fig. 4 compares the turbidimetric data obtained with two concentrations, 1% and 3% (w/v). Increasing the concentration does not necessarily produce more coacervates. The mixtures obtained with 3% (w/v) of polymers, in general, revealed an absorbance identical to that of a concentration with 1% (w/v) of polymers, except for a ratio of 20:1, which there was a clear increase in absorbance. Similar results were encountered by Remunan-Lopez and Bodmeier (1996). They investigated the effect of the total polymer concentration on the coacervate yield for 1:20 chitosan:gelatin and observed that the coacervated phase occupied a greater volume with increasing total polymer concentration, but the dry coacervate yield remained constant.

Increasing the concentration did not affect the optimum pH of coacervation nor the behavior observed at other pHs for all proportions. These results indicate that the quantity of neutral and insoluble complexes formed depends on the proportion of the charges on the system, and the total concentration of polymers evaluated here was insufficient to cause depletion or competition of certain ions.

The concomitant analysis of zeta potential and turbidimetric measurements allowed for the restriction of conditions for complex coacervation analysis applied to produce microparticles.

3.3. Analysis of microencapsulation conditions

After identifying some probable conditions for producing microparticles, two polymer ratios 20:1 and 10:1, two total polymer concentrations (1% and 3%, w/v) and two pH of coacervation: 5.5 and 6.0, were investigated for the production of microparticles with an oil core model. Limonene oil was used for this purpose with a relation of 50% to the wall material.

The use of conventional protocols for the production of coacervated microparticles (Prata, 2006) has failed with all combinations above described.

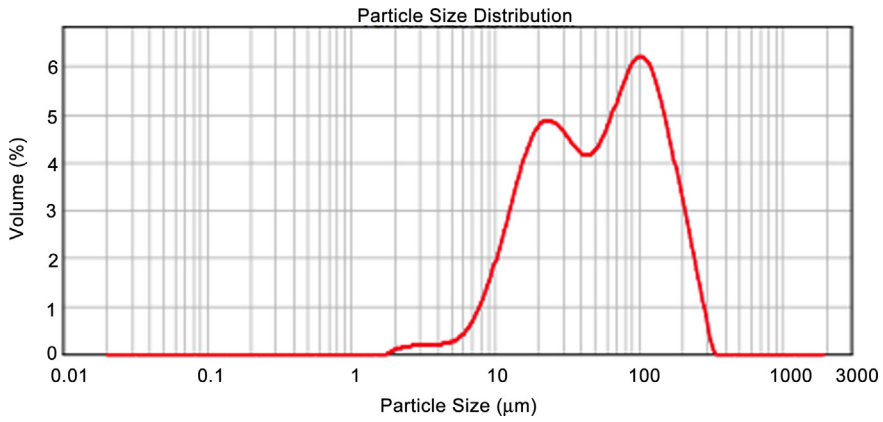

Fig. 5. Size distribution of moist limonene containing chitosan-gelatin microparticles. $D_{3,2}$ = 30.141 μm, span = 2.789.

Many authors, using gelatin and chitosan, presented as part of their methodology the mixture of both polymers prior to emulsification and/or addition of a salt (sodium sulphate) as a promoter of coacervation. Therefore, assuming one condition indicated by the analysis of the zeta potential (pH 6.0 and 10:1 gelatin:chitosan), some modifications based on the specific methodology prescribed in literature were experimentally reproduced. In this context, both emulsification of the oil in the mixture (chitosan plus gelatin) at pH 6.0 and the addition of sodium sulfate (1%, w/v) in the mixture before homogenization and pH adjustment resulted in a gelled mass. Adding sodium sulfate (1%, w/v) to the gelatin-oil emulsion caused the formation of a transparent, but gelled, supernatant and the precipitation of a fibrous slurry, i.e., apparently forming the coacervates. However, in none of the trials reported above, which were carried out systematically and in triplicate, can the formation of particles themselves be seen.

For some applications, it is enough to form complexes as aggregate structures, but even for conditions very similar to that we found in this work, the effective production of isolated microparticles was not verified. Silva and Andrade (2009) found to be the best condition for the production of coacervates the use of high bloom gelatin, chitosan with a low degree of acetylation, ratios of gelatin:chitosan as 10:1 and a pH of coacervation between 4 and 4.5. Hussain and Maji (2008), after gradual addition of sodium sulfate (20%, w/v), verified coacervation at a pH between 7.0 and 8.0 for gelatin:chitosan ratios of 1:1, 2:1 and 1:2. In both works the formation of microparticles were not observed directly. Other authors produced spherical particles by atomization of the coacervate mass. Basu, Kavitha, and Rupeshkumar (2011) emulsified the oil phase on the mixture of polymers (gelatin and chitosan) and adjusted the pH to 5.0. Similarly, Kang et al. (2012) obtained dried coacervated, first produced with a ratio of 1:15 chitosan:gelatin at pH 6.0.

In fact, physical measurements used for estimating the range for complex coacervation established conditions for neutralize charges in the system, but it is impossible to estimate the influence of intuitive variables. It was observed in this work that either formation of a fibrous network or gelation of the system occurred. Because the conditions adopted were far from the pH of gelation of chitosan, there seems to be some problem with the gelatin. Therefore, a rapid complexation between the polymers might avoid the gelation of gelatin. A more diluted system (1%, w/v) with 10:1 gelatin:chitosan, 50 °C and 0.55 g of oil, the pH of chitosan solution was modified for the pH of coacervation (pH 6.0) before joining it to the gelatin emulsion, whose pH is naturally around this value. With this procedure, the limonene-containing microparticles were successfully produced (Fig. 6) and were spherical and much smaller ($D_{3,2}$ = 30 μm) than those obtained by complex coacervation from gelatin and gum Arabic (approximately 100 μm). The efficiency of

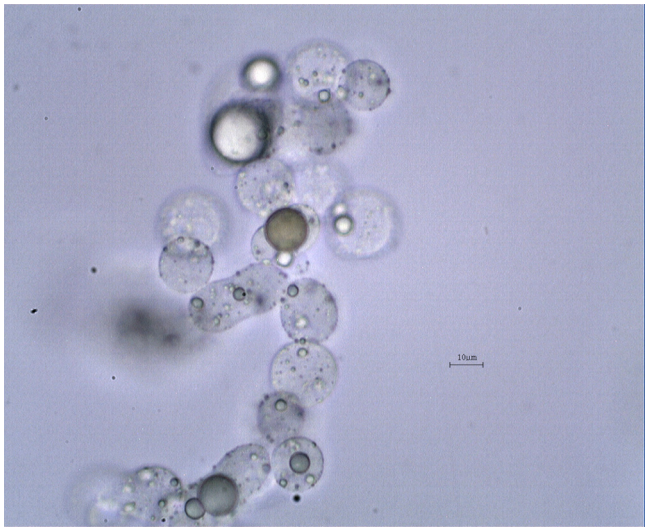

Fig. 6. Optical microscopy of microparticles produced by complex coacervation of gelatin and chitosan. Bar represents 10 μm.

encapsulation of these particles was high (89.6%), considering the total oil in the particle. However, their appearance was oily, which, over time, promotes the formation of agglomerates, as can be seen by the second peak at approximately 100 μm, in Fig. 5. Almost 50% of these particles (d(v,0.5)) presented diameter inferior to 54.9 μm and their homogeneity (span 2.789) was compromised by the tendency to aggregation. These particles also had a high water content (98.5%), and only 57% of the polymers added to the process were precipitated as microparticles.

3.4. Cross-linking and drying of the microparticles

Moist microparticles were cross-linked with sodium tripolyphosphate (Na-TPP) and glutaraldehyde to observe the resistance of the wall during the drying process. Alvim and Grosso (2010) observed that if gelatin-gum Arabic coacervates are not strengthened, they could not remain intact during atomization due to the high pressure/viscous tensile forces imposed.

Cross-linking agents using amine groups from proteins as a substrate are well known, such as transglutaminases and aldehydes including formaldehyde and glutaraldehyde (Prata et al., 2008). However, chitosan has many charged groups and can also interact through electrostatic interactions. Agents that have been widely used for cross-linking chitosans are the genipin (Muzzarelli, 2009; Yuan et al., 2007) and the multivalent anion sodium tripolyphosphate (Basu et al., 2011; Fernandes, De Oliveira,

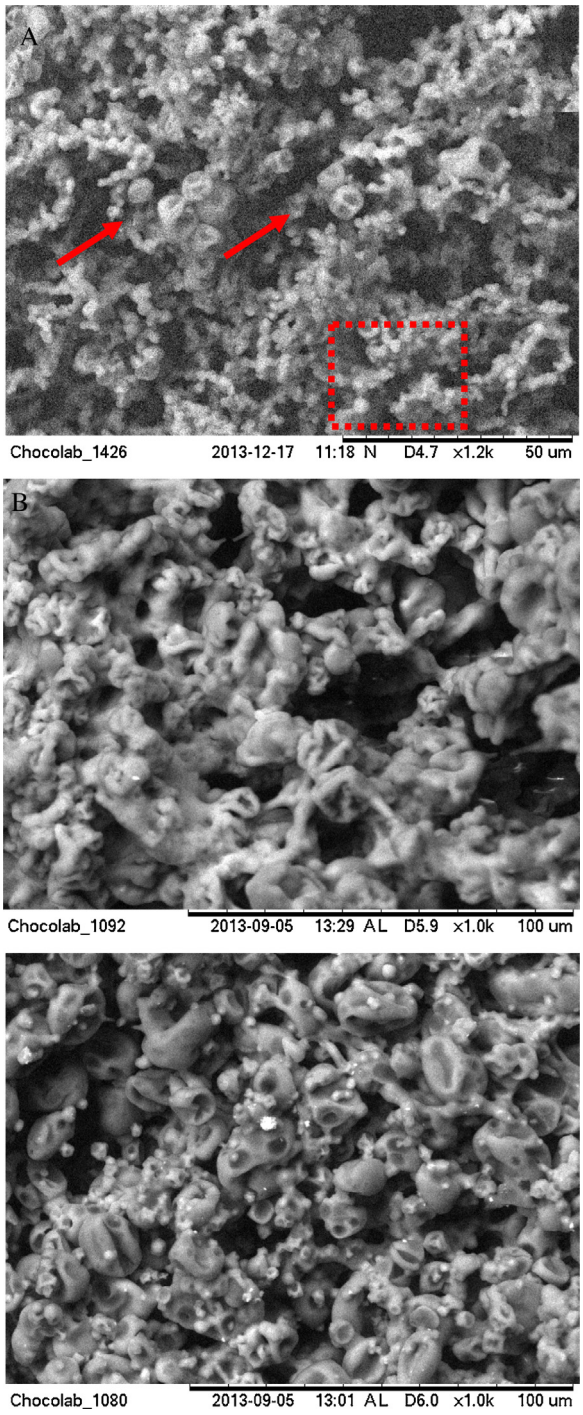

Fig. 7. Electronic microscopy of coacervated microparticles after passing through spray dryer. (A) Without cross-linking, (B) cross-linked with glutaraldehyde, (C) cross-linked with Na-tripolyphosphate. Bar represents 50 μm for A and 100 μm for B and C.

Fatibello-Filho, Spinelli, & Vieira, 2008; Zhao, Shi, & Zhang, 2011). Shu and Zhu (2002) compared the intensity of cross-linking between tripolyphosphate, citrate and sodium phosphate anions and verified that microparticles cross-linked with TPP presented the highest release control and 10 times higher mechanical strength properties than with the other salts.

The effect of cross-linking was assessed by the resistance of the cross-linked microparticles to the spray drying. Some uncross-linked spray-dried microparticles remained intact and spherical (Fig. 7A) indicating that the structure formed by chitosan-gelatin seems to be stronger than gelatin-gum Arabic. In Fig. 7A, the dotted square indicates malformed microparticles, most likely because they could not resist the atomization process. Microparticles cross-linked with glutaraldehyde had a brown color and, after drying, were joined together as a dough mass rather than as individual particles (Fig. 7B). Glutaraldehyde is heat activated and aldehyde residues not bound to amino groups may have polymerized during the drying process. On the other hand, microparticles cross-linked with Na-TPP were easily spray dried, with minimal loss due to adhesion to the wall of the chamber and drag caused by the cyclone. These microparticles were separated and smaller than uncross-linked ones. Before drying, both cross-linked microparticles were spherical and separated.

4. Conclusions

Positively charged polysaccharides such as chitosan have different properties as a function of the pH, lending to the formation of particles by complexation, by gelling or emulsion, which significantly expands the range of structured material formed from this material. The charge analysis of biopolymers on the mixture helps to restrict the experimental range for producing particles by complex coacervation. However, intuitive variables such as the rate of reduction of pH, velocity of agitation, or rate of cooling are not considered in this preliminary analysis and appear as determinants of the shape of the particles. In this work, contrary to polymers whose properties are less dependent on pH, the visual characteristics of the chitosan and gelatin type A mixtures at different pHs did not necessarily correspond to the complexation between biopolymers. This likely occurred because of the insolubility of chitosan in the solutions with a more basic pH. The condition of best match between free charges and turbidity was chosen for production of the particles (10:1 gelatin:chitosan, pH 6.0). However, at these conditions, complexation between the polymers was verified, but the formation of individual particles was not. Prior adjustment of pH of the chitosan solution allowed for the formation of particles. This was attributed to the slow ionization of chitosan during the coacervation process, which does not allow the exposure of chitosan to charges for complexation with gelatin. The particles were then produced with limonene, which exhibited sizes less than 50 μm and an 89.6% encapsulation efficiency. Two cross-linking agents (sodium tripolyphosphate and glutaraldehyde) were tested to allow drying of coacervates spray drying. Of these, the Na-TPP showed better visual results.

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