Carbohydrate Polymers 116 (2015) 292–299

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Production of microparticles with gelatin and chitosan

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ARTICLE INFO

Article history:
Available online 29 March 2014

Keywords:
Complex coacervation
Chitosan
Tripolyphosphate
Limonene
Spray drying

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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http://dx.doi.org/10.1016/j.carbpol.2014.03.056
0144-8617/

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