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Design of chitosan-based nanoparticles functionalized with gallic acid

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ABSTRACT

Active nanoparticles based on chitosan could be applied as a support for the modulation of gallic acid delivery. In this sense, these nanostructures could be employed in different fields such as food, packaging, and pharmaceutical areas. The design parameters of chitosan-based nanoparticles functionalized with gallic acid (GA) were optimized through RSM by means of the analysis of zeta potential (ZP) and percentage encapsulation efficiency (PEE). The nanoparticles were prepared by ionotropic gelation using tripolyphosphate (TPP), at different combinations of chitosan (CH) concentration, CH:TPP ratio and GA. Global desirability methodology allowed finding the optimum formulation that included CH 0.76% (w/w), CH:TPP ratio of 5 and 37 mg_{GA}/g_{CH} leading to ZP of +50 mV and 82% of PEE.

Analysis through QuickScan and turbidity demonstrated that the most stable nanoparticle suspensions were achieved combining concentrations of chitosan ranging between 0.5 and 0.75% with CH:TPP ratios higher than 3. These suspensions had high stability confirmed by means ZP and transmittance values which were higher than +25 mV and 0.21 on average, respectively, as well as nanoparticle diameters of about 140 nm.

FTIR revealed the occurrence of both hydrogen bond and ionic interactions of CH-TPP which allowed the encapsulation and the improvement of the stability of the active agent.

1. Introduction

Research and the application of polyphenols have recently attracted a great interest in the functional foods, nutraceutical, and pharmaceutical industries, due to their potential health benefits to humans. Gallic acid (GA, 3, 4, 5-trihydroxybenzoic acid) is found in abundance in the plant kingdom, especially in tea, grapes, berries, and other fruits, as well as in wine. This polyphenol exhibits a variety of biochemical properties, as antioxidant, antimicrobial, and anti-inflammatory, and it has anticancer and neuroprotectant effects [1]. GA has high reducing potential and scavenging properties toward radical species which explains its antioxidant capacity [2,3]. These functions make it widely used in foods, drugs, and cosmetics [4,5].

The inclusion of polyphenol extracts more concentrated than naturally present in foods is hindered due to off-flavors, poor stability, and reduced solubility. In this context, the use of encapsulation technology has helped overcome these issues by entrapping the phenolic compounds within biopolymer carriers to better control the dosage, improve their stability against oxidation, provide site-specific targeted delivery, mask off-flavors, and improve their miscibility in aqueous food products [6].

The appearance of the nanotechnology has triggered enormous possibilities for obtaining innovative products such as nanosensors [7–9] and applications for a wide spectrum of nutraceutical and food consumption sectors. On the other hand, Patel et al. [10] and Pinheiroa et al. [11] evaluated the abilities of biopolymeric matrices to provide remarkable protection the incorporated compounds against thermo- or photo-degradation.

Chitosan is increasingly favored in various fields of drug delivery. From the chemical point of view, it is a polycationic copolymer consisting of β-(1–4)-linked glucosamine and N-acetylglucosamine units, soluble in an acidic environment due to protonation of the amino groups. The presence of reactive amino groups means that chitosan can be modified easily to create and nanoparticles or porous hydrogels. The resultant positive charge makes it possible to prepare nanoparticles by ionotropic gelation with multivalent anions, such as tripolyphosphate (TPP) [12].

The interaction of chitosan with TPP leads to the formation of biocompatible cross-linked chitosan nanoparticles. The cross-linking density, crystallinity, and hydrophilicity of cross-linked chitosan allow modulation of drug release and extend its range of potential applications in drug delivery based on nanocapsule design. Probably due to a more compact structure, the complex nanoparticles were found to provide additional protection the embedded compounds against heat or light-induced degradation [13]. Chitosan has been reported extensively as an encapsulant for bioactive compounds, such as vitamins and minerals [14–16], catechins [17] and proteins [18].

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Further, antioxidant-chitosan conjugates showed diverse bioactivities, including antioxidant, anticancer, and inhibitory effects on digestive enzymes, β-secretase, and food-borne pathogens [4].

Experimental design consists of a set of experiments, in which the levels of all contributing variables are changed simultaneously in a systematic manner. The traditional one-factor at a time (OFAT) technique used for optimization does not take into account interactions between the factors and provides less information about the variability of the response [19]. In contrast, the experimental design is a valuable tool for measuring interactions among factors and for the prediction of optimal conditions, being less expensive and less time-consuming [20]. Another strong point of using statistical optimization is that no complex calculations are required to analyze the resulting data [21].

A central composite design (CCD) consists of embedded factorial design, star points to estimate the curvature, and center points to evaluate the experimental reproducibility. The center points are selected to obtain several properties, such as rotatability or orthogonality, in order to fit the quadratic polynomials [22]. This statistical tool significantly reduces the number of empirical experiments that are necessary to identify a mathematical trend in the experimental design, facilitating determination of the optimum level of variable factors required for a given response [23]. The CCD is one of the designs most used, due to its flexibility. It has been successfully applied to the optimization of many bioprocesses [24,25].

Among different types of experimental design, the response surface methodology (RSM) has become the standard approach for many of the experiments carried out for optimization purposes. RSM is mostly concerned with approximating a complex, unknown function with a polynomial, usually either a first-order model or a second-order model. Consequently, designs for fitting these models are of considerable interest and allow estimating interaction and even quadratic effects and therefore, give us an idea of the shape of the response surface.

There is hardly any information about the optimization of the nanoencapsulation process of food preservatives by using chitosan matrices. In this sense, this study seeks to contribute to the food preservation by means useful information about tailor-made functionalized nanoparticles. Therefore, the objectives of the present work were: to a) optimize design parameters for encapsulating GA in chitosan particles by using RSM focusing on food applications, b) evaluate the effects of key processing variables (chitosan concentration, CH:TPP ratio and GA concentration) on the yield of the functionalizated nanoparticles through zeta potential and percentage encapsulation efficiency, c) evaluate the physical suspension stability by means of QuickScan and turbidity methods, d) obtain information concerning the way by which the components of the particle system interact among them through FTIR.

2. Materials

Chitosan (CH) from crab shells with a minimum deacetylation degree of 85% and a molecular weight of 4.8×10^5 Da was purchased from Polymar Ciência e Nutrição (Fortaleza, Brazil). Analytical grade sodium tripolyphosphate (TPP), gallic acid (GA) and acetic acid were supplied by Anedra (Buenos Aires, Argentina).

3. Methods

3.1. Preparation of nanoparticles

A stock chitosan solution of 2% (w/w) was prepared by solubilization in 2% (v/v) acetic acid solution at 20 °C under continuous stirring for 24 h approximately, followed by a vacuum filtration to eliminate insoluble materials. Cross-linking agent sodium tripolyphosphate was dissolved in distilled water under constant stirring at the concentration of 2% (w/v) to prepare the stock TPP solution. CH and TPP solutions of different concentrations were obtained from the stock solutions consistent with the concentrations summarized in Table 1. Afterwards, the corresponding amounts of GA were added to the carrier solutions until reaching the final concentration (expressed as mg per gram CH) as shown in Table 1.

The CH nanoparticles were obtained by inducing the ionotropic gelation of a chitosan solution using TPP as counter ion, following the method described by Calvo et al. [26] with some modifications. Nanoparticles were formed spontaneously by dropwise addition of a TPP solution into a solution containing CH and GA. Once this process was completed, the obtained suspensions were homogenized at 13,500 rpm for 10 min by means of an Ultraturrax T-25 (Janke & Kunkel, IKA-Labortechnik, Germany).

From here onwards, the chitosan nanoparticles charged with GA will be called F_1, F_2, …, F_n, with $n = 15$.

3.2. Percentage encapsulation efficiency (PEE)

To determine the percentage encapsulation efficiency, the synthesized nanoparticles were separated from the suspension by centrifugation at 20.000 rpm for 20 min (Beckman Coulter Optima L-100XP Floor Centrifugation System, California, USA). Samples were filtered using 0.45 μm sterile nylon (Millipore, Bedford, MA, USA) and the concentration of GA was quantified by using a Spectrophotometer DU 650 (Beckman, USA) [5]. The concentration of the active agent in both the supernatant and the precipitate was calculated according to a calibration curve, prepared by measuring the absorbance at $\lambda = 269$ nm (determined as the maximum of absorbance of the active compound) of known concentrations of GA. The calculated concentration was then multiplied by the volume of the supernatant to determine the total amount of GA present in the supernatant. All the measures were carried out by triplicate.

The PEE percentage was determined as follows:

$$\mathrm{PEE} = \frac{W_i - W_s}{W_i} \times 100 \quad (1)$$

where, W_i is the mass of GA used for preparation of the nanoparticles and W_s is the mass of GA measured in the supernatant.

3.3. Zeta potential, particle sizes and polydispersity index

Zeta potential and particle hydrodynamic size of the nanoparticle suspensions were determined by dynamic laser light scattering by using a Zetasizer Nano-ZS instrument (Malvern Instruments, Worcestershire, England) provided with a He—Ne laser beam operating at 633 nm at a fixed scattering angle of 173° and a digital correlator Model ZEN3600. ZP was determined by measuring the direction and velocity of droplet movement in a well defined electric field. Prior to the analysis, the index of refraction, determined by using a digital refractometer (Atago, USA), and the conductivity, measured with a pH/conductivity meter (Mettler Toledo Urdorf, Switzerland), were evaluated in each and every suspension.

The zeta potential was reported as the average and standard deviation of measurements made on two samples, performing five determinations per sample.

Polydispersity index (PDI) is a parameter to define the nanoparticle size distribution obtained from photon correlation spectroscopic analysis. It is a dimensionless number extrapolated from the autocorrelation function and ranges from a value of 0.01 for monodispersed particles up to values of 0.5–0.7. Samples with very broad size distribution have PDI values >0.7 [27,28].

3.4. Response surface methodology

The major factors affecting the particle size and percentage encapsulation efficiency of nanoparticles were the biopolymer concentration of CH, the active agent concentration GA and the ratio of the biopolymer to the crosslinking agent CH:TPP.