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Factorial design as tool in chitosan nanoparticles development by ionic gelation technique

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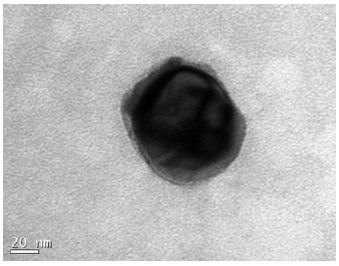
HIGHLIGHTS

- This work shows how to obtain chitosan nanoparticles with properties desired.
- The formulation was optimized using a 2^3 factorial design.
- Nanoparticles were obtained with size <90 nm.
- Nanoparticles were characterized by FTIR, TGA, EDX and TEM analysis.
- Sonication is an important step, otherwise, the nanoparticles agglomerate again.

GRAPHICAL ABSTRACT



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ABSTRACT

Since there are different kinds of chitosan, obtained from different sources of chitin and routes, it is very important to study which are the best conditions in order to obtain nanoparticles with desired size and superficial charge. The aim of this paper was to demonstrate to the scientific community how to obtain chitosan nanoparticles (nanoCTS) with these properties. Ionic gelation technique was applied to form nanoparticles through linkages between tripolyphosphate (TPP) and chitosan (CTS). The formulation was optimized using a 2^3 factorial design to analyze the effect of the pH, CTS:TPP ratio and acetic acid concentration in size and charge of the particles. The best formulation was characterized by FTIR, TGA, EDX and TEM analysis. The formation of nanoCTS depended mainly on ratio of CTS:TPP. Nanoparticles were obtained with excellent conditions for the application in pharmaceutical studies: average diameter of 76.2 nm and zeta potential of 32.6 mV.

1. Introduction

Chitosan is a polysaccharide composed of β(1→4) linked units of N-acetyl-D-glucosamine and D-glucosamine forming a long and linear chain [1]. This polymer can be naturally found in the cell wall of certain groups of fungi, particularly zygomycetes [2] and also can be obtained from partial deacetylation, by alkaline or enzymatic hydrolysis, of chitin which is naturally found in insects, arachnids, and crustaceans.

Since Bodmeier et al. [3] reported that small particles can be obtained by dripping a solution of CTS onto a solution of TPP, many researchers have explored this property in pharmaceutical studies [4–9].

Due to the advantageous biological properties of CTS, such as wound healing effect [9], antimicrobial activity [10], low toxicity,

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biocompatibility, biodegradability [11], cationic properties and bioadhesive characteristics [12], nanoCTS have been extensively applied.

NanoCTS have been widely used in pharmaceutical applications mainly as drug carrier [4,13] and for DNA delivery [14,15]. The nanoparticles can easily penetrate through capillary and epithelial tissue and this allows an efficient delivery of therapeutic agents, such as drugs, to target sites in the body. According to Gan et al. [16], smaller size nanoparticles (~100 nm) demonstrated more than 3-fold greater arterial uptake compared to larger nanoparticles (~275 nm).

CTS has antibacterial activity that is associated with the positive charge of the amino groups, which can bind to the bacterial cell surface and interfere with normal functions of the membrane, inhibiting their growth [17]. When CTS is in nanoparticles form, exhibits higher antibacterial activity than CTS powder because the polycationic nanoCTS has higher surface area and charge density and can interact to a greater degree with the negatively charged surface of the bacterial cell [18]. Therefore, the antibacterial activity of nanoCTS has been explored by many researchers [19–21].

Different methods for development of nanoCTS can be found in literature as: ionic gelation [7,12,22], emulsion [23], synthesis with carboxymethyl cellulose [24], formulations using glutaraldehyde [25], synthesis with alginate [26], coacervation [27], reverse micellar [28] and polymerization with poly(hydroxyethyl methacrylate) [29].

Ionic gelation technique presents the following advantages over other methods: the nanoparticles are obtained spontaneously under mild control conditions without involving high temperatures, organic solvents, or sonication [30] and the TPP is a multivalent polyanion, with low toxicity and cost, unlike other cross-linkers, it presents no severe constraints of handling and storage. After adding TPP solution, nanoparticles form immediately through inter and intramolecular linkages created between TPP phosphates and CTS amino groups [4]. Generally, in this method nanoparticles are prepared by addition of TPP solution (pH 7–9) into an acidic solution (pH 4–6) of chitosan [31].

The particle size and surface zeta potential can be manipulated by variation of the development conditions such as CTS:TPP ratio, CTS concentration and pH solution [16,32,33]. Since there are different kinds of CTS, with distinct deacetylation degrees and molar weights, it is very important to study, with an experimental design, which is the best condition in order to obtain nanoparticles with size and superficial charge appropriate.

Optimization of experiments, such as those used in nanoparticles development, can lead to useful savings of scientific resources. Factorial experimental designs are commonly used to optimize experiments and discover which factors influence the outcome of the experiment and what levels of these factors lead to a test with the better response [34].

There are a few works in the literature [35,36] that demonstrate how to obtain nanoCTS by ionic gelation method with desired characteristics using a factorial design, however, these works present nanoCTS with diameter greater than 100 nm.

This study aims to investigate the influence of acetic acid concentration, pH and ratio of CTS:TPP in the size and zeta potential of the nanoparticles obtained through ionic gelation. The nanoparticles formulation was optimized using a 2^3 factorial design (FFD), with 8 experiments (in duplicate), to analyze the effects of the three selected factors.

2. Experimental

2.1. Materials

CTS with deacetylation degree of 81.9% and molar mass of 111.01 kDa was supplied by Purifarma (Brazil), sodium tripolyphosphate and potassium bromide from Sigma–Aldrich (USA), sodium hydroxide and glacial acetic acid were obtained from Synth (Brazil).

2.2. Analysis of different ratios of CTS:TPP

NanoCTS were produced according ionic gelation technique described by Calvo, Remuñán-López, Vila-Jato and Alonso [30]. CTS solution, with concentration of 2.5 mg mL^{-1}, was prepared by dissolving the polymer with sonication in 1% (w/v) acetic acid solution until the solution was transparent and the pH was adjusted to 4.5 with NaOH 0.1 M. Tripolyphosphate was dissolved in deionized water at the concentration 1 mg mL^{-1}. A volume of 10 mL of TPP solution was mixed in a different volume of CTS solution to achieve ratios of CTS:TPP of 2:1 until 6:1. The nanoparticle suspensions were gently stirred for 1 h at room temperature before being subjected to further analysis.

2.3. Factorial design

The influence of 3 different parameters on the nanoCTS properties was evaluated by using a 2^3 factorial design composed of 3 factors that were set at two levels each. Two different CTS solutions were prepared by dissolving 2 mg mL^{-1} of polymer, with sonication, in 1% (w/v) and 0.1 M acetic acid solutions until the solutions were transparent. Once dissolved, both CTS solutions were divided into two equal volumes but with different pH: 4.4 and 4.6. TPP was dissolved in deionized water at the concentration of 1 mg mL^{-1}. In tests with ratio of CTS:TPP of 3:1, 10 mL of the TPP solution were added to 15 mL of the CTS solution and in the experiments with the ratio of 3:0.8; 8 mL of the TPP solution were added to the CTS solution (15 mL). The eight different formulations of the experimental planning were kept under magnetic stirring, at room temperature, for 1 h.

2.4. Nanoparticles characterization

2.4.1. Size, zeta potential and polydispersity index (PDI)

The average size, zeta potential and polydispersity index of the nanoparticles were measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Three batches of each formulation were analyzed ($n = 3$).

2.4.2. Transmission electron microscopy (TEM)

The morphological examination of nanoCTS was conducted by transmission electron microscopy (TEM) (JEM-1011, JEOL, Japan) using an acceleration tension of 100 kV. The samples were placed on carbon coated copper grid for TEM observation.

2.4.3. Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopy (IR) measurements were carried out with CTS and nanoCTS. Prior to the assay, KBr was gently triturated with the dried samples (2% of polymer) and compressed into discs. FTIR spectra were obtained using IR Prestige-21 spectrometer (Shimadzu, Japan) in the 400–4000 cm^{-1} region at room temperature.

2.4.4. Energy dispersive X-ray spectroscopy (EDX)

EDX analysis was performed on CTS and nanoCTS. The dried samples were stuck on stubs using double-sided tape. Then, the samples were coated with a gold film and analyzed with a field-emission-gun scanning electron microscope (FEG-SEM) (JSM-6701F, JEOL, Japan) coupled with an energy dispersive-X detector (EDX).