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Chitosan-tripolyphosphate nanoparticles: Optimization of formulation parameters for improving process yield at a novel pH using artificial neural networks

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ABSTRACT

At a novel pH value of the polymeric solution (6.2), variable chitosan (Cs) and sodium tripolyphosphate (TPP) concentrations and mass ratios were optimized to improve the process yield without undesirable particle flocculation. Prepared formulations were characterized in terms of particle size (PS), zeta potential (ZP) and percentage yield (% yield). Artificial neural networks (ANN) were built up and used to identify the parameters that control nanoparticle (NP) size and yield, in addition to being tested for their ability to predict these two experimental outputs. Using these networks, it was found that TPP concentration has the greatest effect on PS and% yield. The most optimum formulation was characterized by a notable process yield reaching 91.5%, a mean hydrodynamic PS 227 nm, ZP + 24.13 mv and spherical compact morphology. Successful Cs-TPP interaction in NP formation was confirmed by both Fourier transform-infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC). This study demonstrated the ability of ANN to predict not only PS of the formed particles but also NP% yield. This may have a great impact on Cs-TPP NPs preparation and can be used to customize the required target formulations.

1. Introduction

Chitosan nanoparticles (Cs NPs) offer numerous advantages qualifying them to be promising drug delivery systems [1]. Chitosan (Cs) is a biodegradable and biocompatible polymer composed of β1 → 4 linked glucosamine and *N*-acetyl glucosamine monomer and it is manufactured commercially on a large scale by the *N*-deacetylation of the abundant biopolymer, chitin; isolated from the exoskeleton of crustaceans such as crabs and shrimps [2]. Cs is a hydrophilic polycationic polymer at acidic pH, which in the presence of polyanions such as pentasodium tripolyphosphate (TPP) can spontaneously gel into submicron sized particles. This famous ionotropic gelation method is relatively simple, mild and avoids the use of organic solvents and high temperatures. Since first described by Calvo et al. [3]. Cs ionotropic gelation has been extensively studied en route for obtaining nanocarrier systems with tunable geometries, positive surface charge and a good capacity of drug encapsulation.

However, limited body of literature incorporated results on the production method yield [4], possibly because it is relatively low. Nowadays, Cs-based nanocarriers are under advanced preclinical and early clinical development as reported by Garcia et al. [1]. Therefore, enhancement of Cs-TPP NP production yield would, indeed, be important for increasing the productivity of this preparation method when applied on the industrial scale. Moreover, more NP production means more nanovehicles available in the formulation which would reflect on improving drug loading efficiency. In a recent study [5], our team demonstrated the enhancement of yield and diminution of Cs-TPP Nps when both polymer and cross-linker solutions were used at elevated pH conditions and lowest possible mass ratios. Accordingly, in the present work, our team attempts to use a novel Cs solution pH 6.2 to favor the preparation of electropositive Cs-TPP nanosuspension with maximized NP yield.

To our best knowledge, this is the first study that investigates the use of Cs solution at a pH 6.2 for the preparation of Cs-TPP NPs. However, this pH value is considered critical; the reduced charge

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density on polymer chains ($pK_a = 6.5$) might render the polymer susceptible to aggregation. Accordingly, it was important, in this study, to evaluate the effect of increasing Cs solution pH and concentration on NP productivity and properties, but as well important was to identify the concentration and amount of cross-linker used favoring the preparation of a monodisperse NP population. Cs-TPP NP preparation is a multivariate process; the ultimate characteristics of CsNPs are influenced by the various formulation parameters. In recent years, artificial neural network (ANN) has been introduced in pharmaceutical research as a powerful tool for modeling and analyzing complex multivariate processes [6].

The architecture of ANNs is biologically inspired to simulate the way in which the human brain processes information ANNs are generally presented as machine learning computer programs composed of interconnected processing units called "neurons" which send messages to each other. In most cases identical neurons are interconnected in at least three separate layers; the input data are received in the input layer and the weighted sum of all inputs is computed in the hidden layer(s), with the output results reported through the output layer [7]. The connections have numeric weights that can be tuned based on training experience, making neural nets adaptive and capable of learning. Nowadays, ANN is successfully applied in the analysis and modeling of complex multivariate nonlinear relationships frequently encountered in pharmaceutical research and that are difficult to model using classical statistical methods [8,9]. With the added benefits of short computing time and high potential of quality adaptive performance, these artificial intelligence tools have been successfully used in the design of controlled release delivery systems [10], in the evaluation of in vitro-in vivo correlation for dry powder inhalers [11], also used for improving the understanding of the process of formation of nanoemulsion [6] and the determination of the factors controlling the particle size and entrapment efficiency of noscapine in PEG/PLA nanoparticles [12].

The aim of this study was to characterize Cs-TPP NPs formulated at novel Cs solution pH 6.2, when Cs and TPP concentrations and mass ratios were used as experimental variables by applying ANN in identifying the factors specifically influencing NP yield and size.

2. Materials and methods

2.1. Materials

Low molecular weight (Mw) Cs (75–85% degree of deacetylation, viscosity 20 to 300 cP, average Mw ~ 50 kDa, Cat. No.: 448869) and pentasodium tripolyphosphate (TPP) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH) was supplied from El-Nasr Pharmaceutical Chemicals (Egypt). Deionized water used throughout the research work was produced using a Milli-Q Gradient A10 System. All chemicals and reagents were used as received without further purification.

2.2. Cs solubility assessment at pH 6.2

The solubility of Cs was assessed primarily at such critical pH 6.2 by visual observation for solution clarity and any aggregates formation [13]. Additionally, a quantitative solubility test was performed according to the method adopted by [14] for the highest examined Cs concentration (0.3% w/v) as follows:

A weighed sample was dissolved in 1% acetic acid and stirred at room temperature till complete dissolution, and then the sample was filtered (0.22 μm membrane filter), dried and weighed. The solubility was determined by the following equation:

$$\text{Solubility\%} = \left[\frac{\text{weight of soluble part}}{\text{total weight of sample}}\right] \times 100$$

2.3. Preparation of Cs-TPP NPs

Cs-TPP NPs preparation followed the ionotropic gelation method was first described by Calvo et al. [3]. Low molecular weight Cs was dissolved in 1% w/v acetic acid at concentrations of 0.1, 0.2 and 0.3% w/v and kept under magnetic stirring until complete dissolution. Elevation of Cs solution pH to 6.2 was performed by the addition of appropriate volumes of 1 N NaOH, such pH value maintains Cs in its soluble form at all examined concentrations. TPP solution was prepared at different concentrations: 0.01, 0.02, 0.03, 0.04 and 0.05% (w/v). Both solutions were filtered through a 0.22 μm pore size filter (Millipore, Billerica, USA). The Cs-TPP NPs were formed spontaneously by the dropwise addition of TPP solution onto Cs solution using 3 variable mass ratios (5:1, 7:1 and 9:1) of Cs and TPP, respectively. The gelation process was carried out under constant magnetic stirring (Yellow line MAG HS7, IKA, Germany) (500 rpm) at room temperature.

2.4. Characterization of Cs-TPP NPs

2.4.1. PS and ZP determination

Mean hydrodynamic PS, PDI and ZP measurements were performed on all Cs-TPP NP samples at a temperature of 25 ± 0.5 °C using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK). For PS analysis, a dynamic light scattering (DLS) technique was applied while Laser Doppler Anemometry (LDA) was used for ZP measurements. All samples were analyzed in suspension immediately after preparation in order to avoid changes in PS and charge due to Ostwald ripening or particle growth [15]. The mean of three measurements was calculated for PS, PDI or ZP.

2.4.2. Determination of Cs-TPP NP% yield

Being the best separation condition investigated giving the highest amount of particle recovery, the centrifugation technique was applied [16] using a cooling micro-centrifuge (Hermle Labortechnik GmbH, Model Z216 MK, Germany) adjusted at a speed of 15000 rpm under cooling conditions at 4 °C for 2 h. It is worthy to note that such centrifugal conditions applied for NP separation had no influence on the solubility of the individual Cs solution adjusted to pH 6.2, within the inspected concentration range. This was recognized by the absence of Cs precipitation and pellet formation. The pellets, however, formed from Cs-TPP NP formulations were lyophilized then weighed. A 50 ml volume of Cs-TPP NP formulation was most suitable for the evaluation of% yield by this gravimetric method and it was calculated as follows:

$$\%\text{Yield} = \left(\frac{W_n}{W_t}\right) \times 100$$

where W_n is the total weight of Cs-TPP NPs recovered and W_t is the total weight of Cs and TPP used in the formulation observation.

2.4.3. Morphological characterization

Morphological examination of the optimized Cs-TPP NPs was performed by High resolution-transmission electron microscopy (HR-TEM) (JEOL JEM-2100, Japan) with an acceleration voltage at 200 kV. All samples for TEM analysis were prepared by allowing a single drop of NP suspension to dry at room temperature on a carbon-coated copper meshwork without being stained [17,18].

2.4.4. Fourier transform-infrared (FT-IR) spectroscopy

FT-IR spectrophotometer (Nicole 6700, thermoscientific, USA) was employed to record IR spectra (range 4000–400 cm^{-1}) of both