Colloids and Surfaces B: Biointerfaces 146 (2016) 136–143

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Multivariate analysis for the optimization of polysaccharide-based nanoparticles prepared by self-assembly

Sara Pistone*, Dafina Qoragllu, Gro Smistad, Marianne Hiorth

SiteDel Group, School of Pharmacy, University of Oslo, P. O. Box 1068, Blindern, 0316 Oslo, Norway

ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form 2 May 2016
Accepted 18 May 2016
Available online 19 May 2016

Keywords:
Polysaccharide nanoparticle
Sodium alginate
Ionic crosslinking
Monodisperse
Zinc chloride
Polydispersity index

ABSTRACT

Polysaccharide-based nanoparticles are promising carriers for drug delivery applications. The particle size influences the biodistribution of the nanoparticles; hence size distributions and polydispersity index (PDI) are critical characteristics. However, the preparation of stable particles with a low PDI is a challenging task and is usually based on empirical trials. In this study, we report the use of multivariate evaluation to optimize the formulation factors for the preparation of alginate-zinc nanoparticles by ionotropic gelation. The PDI was selected as the response variable. Particle size, size distributions, zeta potential and pH of the samples were also recorded. Two full factorial (mixed-level) designs were analyzed by partial least squares regression (PLS). In the first design, the influence of the polysaccharide and the crosslinker concentrations were studied. The results revealed that size distributions with a low PDI were obtained by using a low polysaccharide concentrations (0.03–0.05%) and a zinc concentration of 0.03% (w/w). However, a high polysaccharide concentration can be advantageous for drug delivery systems. Therefore, in the second design, a high alginate concentration was used (0.09%) and a reduction in the PDI was obtained by simultaneously increasing the ionic strength of the solvent and the zinc concentration. The multivariate analysis also revealed the interaction between the factors in terms of their effects on the PDI; hence, compared to traditional univariate analyses, the multivariate analysis allowed us to obtain a more complete understanding of the effects of the factors scrutinized. In addition, the results are considered useful in order to avoid extensive empirical tests for future formulation studies.

1. Introduction

Alginate is a natural polysaccharide extracted from brown algae that is widely used in several different fields [1–3]. FDA classifies alginate among the generally regarded as safe (GRAS) food additives, and purified alginate is commonly used also for biomedical and pharmaceutical applications [4–6] due to its biocompatibility, biodegradability and low toxicity.

Alginate is a linear unbranched copolymer composed of β-D-mannuronic units (M) bound with units of the C-5 epimer α-L-guluronic acid (G) through (1 → 4) linkages [7] (Fig. 1). M and G residues, when deprotonated, are negatively charged and in this form they can bind divalent or multivalent cations, thus creating three-dimensional ionotropic gel networks [7,8]. The gel-forming properties make alginate an interesting compound for drug delivery, in particular for formulations with sustained and controlled release [9–11]. In fact, the alginate gel network can create a viscous barrier that controls the diffusion of the entrapped molecules depending on the pH and ionic strength of the environment [12,13]. Moreover, alginate has mucoadhesive properties that can also improve the bioavailability by facilitating prolonged delivery at the site of administration [14].

Depending on the desired application, alginate gels can be prepared in different forms, such as block gels, films, beads, fibers, microparticles, and nanoparticles. Alginate nanoparticles have mainly been prepared through two methods; the alginate-in-oil emulsification method and the self-assembly method [15]. The self-assembly method is particularly interesting since organic solvents are avoided and mild preparation conditions are used allowing for encapsulation of sensitive material [16–19].

In the self-assembly method, solutions of one or two different cationic crosslinkers are added to an alginate solution under controlled conditions while mixing. This can be followed by further processing, such as sonication and centrifugation. Also other polysaccharide nanoparticles can be prepared through the self-assembly method, such as nanoparticles made with chitosan

* Corresponding author.
E-mail addresses: sara.pistone@farmasi.uio.no (S. Pistone), ina_790@hotmail.com (D. Qoragllu), gro.smistad@farmasi.uio.no (G. Smistad), marianne.hiorth@farmasi.uio.no (M. Hiorth).

http://dx.doi.org/10.1016/j.colsurfb.2016.05.055
0927-7765/

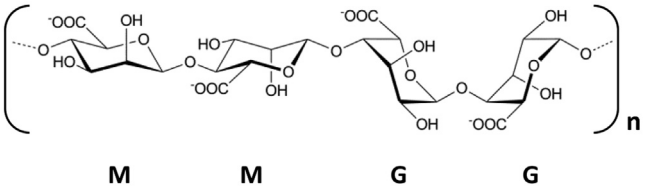

Fig. 1. Example of molecular structure of the alginate chain. Monomers of mannuronic acid (M) and guluronic acid (G) are bound to each other through (1 → 4) linkages.

or pectin [20,21]. This method of preparation of polysaccharide nanoparticles involves many factors that can affect important characteristics of the nanoparticles. For example, the concentration of the polysaccharide solution, the molecular weight of the polysaccharide and the preparation conditions can affect the size; the crosslinker concentration can modify the polydispersity and the zeta potential; the pH and the ionic strength of the solvent can change the compactness of the particles [17,21–28]. Therefore, by tuning different factors, it is possible to achieve the desired characteristics of the nanoparticles.

Nevertheless, empirically finding the optimal levels for all the factors is seldom an easy task since many factors are involved. Moreover, the variation of one factor at a time as in univariate approaches could be limiting since possible interactions between the factors can be hidden. Multivariate analysis can be a valuable tool for analyzing the data when more than one factor is involved in the process [29,30]. In particular, the partial least squares regression analysis (PLS) is a statistical method that can be used to determine the interactions between the factors and their square effects, the significant factors that modify the response, and the trends of the effects on the response. In addition, PLS can also predict the values of the factors that, based on the model, can provide the optimal response value [31].

In a recent study [26], we have successfully prepared stable alginate nanoparticles by self-assembly using zinc as the crosslinker without the need of additional polycations, which are commonly used for stabilization. In the present study, a PLS was performed for the first time for the investigation of important factors influencing the characteristics of polysaccharide nanoparticles prepared by self-assembly. The formulation factors of the new nanoparticulate system made with alginate were optimized by the use of factorial design and multivariate evaluation for providing nanoparticles suitable for drug delivery purposes with a low polydispersity and monomodal size distributions. Two different designs were investigated. The first design was used to study the factors crosslinker (zinc) concentration and polysaccharide concentration. The second design was used to investigate the possibility of producing nanoparticles at the highest level of alginate concentration, since a high polysaccharide concentration can be preferred for drug delivery formulations. This was examined by varying the ionic strength of the solvent and the crosslinker concentration. The response variable investigated in the multivariate analysis was the polydispersity index (PDI) of the samples. In addition, the average size, the size distributions, the zeta potential and the pH were recorded.

2. Materials and methods

2.1. Materials

Water-soluble alginate (sodium alginate, Protanal LF 10/60) was manufactured by FMC BioPolymer (Norway). The alginate was purified prior to utilization and characterized for a viscosity average molecular weight of 147 kDa [26]. The alginate content of G stated by the manufacturer was 65–75%. Zinc chloride (purity ≥ 98.0%) was supplied from Merck (Germany), and sodium chloride (purity 99.9%) was supplied from VWR BDH Prolabo (USA). The water used throughout the study was purified by deionization and filtration through a Millipore Milli-Q system with 0.22 μm Millipak® 40 filter (MilliporeTM, Ireland).

2.2. Preparation of the nanoparticles

The ionic gelation method used for the preparation of the alginate-zinc nanoparticles has previously been reported in detail [26]. In short, 15 g of zinc solution were dripped into 60 g of alginate solution with a constant flow (9.3 ml min^{-1}) and a constant magnetic stirring (600 rpm) at room temperature. Both solutions were prepared in the same solvent. In order to avoid dust contamination, the solutions were previously filtered and the vials were rinsed with filtered water. All the preparation parameters and equipment employed for the preparation were the same as described previously [26]. The various concentrations of the zinc and the alginate of the samples were obtained by keeping constant the mass of the initial alginate and zinc solutions employed and varying their concentrations. During the preparation, the samples were stirred for ten minutes, and then stored overnight at room temperature for stabilization before characterization. No further purification or alteration was carried out before characterization.

2.3. Characterization of the nanoparticles

2.3.1. Dynamic light scattering (DLS)

A Zetasizer Nano ZS (Malvern Instruments Ltd., UK) was used to conduct DLS measurements. The instrument irradiated the samples at 25 °C with a red light laser (λ = 633 nm), and the scattered light was measured with backscatter detection at a scattering angle of 173°. The intensity fluctuations of the scattered light generated an autocorrelation function. Then a general purpose fitting method for the autocorrelation function and the Stokes-Einstein equation were employed by the Zetasizer Software (version 6.20) to calculate the hydrodynamic diameters of the particles. The viscosity and the refractive index at 25 °C of pure water were used as constant parameters in the calculations, independently from the salinity of the solvent. The samples were measured without further dilution. The obtained data concerning the particle size were the intensity-based size distribution plots, the PDI and the intensity weighted mean hydrodynamic diameter expressed as the z-average. However, as stated in the Zetasizer user manual, the z-average is reliable only when the PDI is lower than 0.5, even for comparative purposes [32]. The data reported are the average of three measurements on the same sample aliquot.

2.3.2. Zeta potential

The Zetasizer Nano ZS was also employed to measure the zeta potential of the particles at 25 °C by laser Doppler electrophoresis technique. The Smoluchowski approximation for Henry equation allowed for the calculation of the zeta potential (ζ): $U = \varepsilon\zeta/\eta$, where U is the electrophoretic mobility of the particles measured by the instrument when an electric field is applied. For the constants ε and η (the dielectric constant and the viscosity of the solvent, respectively), the values of pure water at 25 °C were used independently from the ionic strength of the solvent in the sample. The samples were measured without further dilution. The data reported are the average of five measurements on the same sample aliquot.

2.3.3. pH

A 744 Metrohm pH meter (Metrohm, Switzerland), calibrated between pH 4 and 7, was used to measure the pH of the samples at room temperature.