International Journal of Biological Macromolecules 95 (2017) 564–573

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Designing chitosan-tripolyphosphate microparticles with desired size for specific pharmaceutical or forensic applications

Ezzeddin M.A. Hejjaji[a,b], Alan M. Smith[b], Gordon A. Morris[a,*]

[a] Department of Chemical Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield HD1 3DH, UK
[b] Department of Pharmacy, School of Applied Sciences, University of Huddersfield, Huddersfield HD1 3DH, UK

ARTICLE INFO

Article history:
Received 1 July 2016
Received in revised form
17 November 2016
Accepted 23 November 2016
Available online 25 November 2016

Keywords:
Chitosan-tripolyphosphate microparticles
Ionic gelation
Experimental design

ABSTRACT

Chitosan (CS) is a natural cationic polymer obtained by the partial *N*-deacetylation of chitin. Chitosan microparticles can be prepared by cross-linking with tripolyphosphate (TPP) *via* the ionic interaction between positively charged amino groups (CS) and negatively charged counter ions (TPP). This can be controlled by the charge density of CS and TPP, which depend on the pH and ionic strength of the solution. The purpose of this study is to investigate the combined effects of three independent variables (pH, ionic strength and CS:TPP ratio) on three important physico-chemical properties (viscosity, zeta potential and particle size) during the preparation of microparticles. CS:TPP microparticles were prepared using experimental design and equations were generated and used to predict relative viscosity, zeta potential and particle size under different conditions. This gives us the ability to design tuneable CS-TPP microparticles with desired size for specific pharmaceutical or forensic applications *e.g.* latent fingerprint visualisation.

1. Introduction

Chitosan refers to a family of linear copolymer polysaccharides consisting of β(1-4)-linked 2-amino-2-deoxy-D-glucopyranose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) units with different fractions of acetylated units [1]. An acetyl group may be present on some units (*N*-acetyl-D-glucosamine), which determines the degree of deacetylation (DD). Moreover, the DD of commercial chitosan is approximately 66–95%, and the molecular weight (M_W) approximately 10,000–1,000,000 g/mol [2]. The structural units of chitosan have one reactive primary amino group ($-NH_2$) on the C-2 position of each D-glucosamine unit, and two reactive free hydroxyl groups (—OH) for each C-6 and C-3 position building unit (glucosamine and *N*-acetyl-D-glucosamine). These groups (both amino and hydroxyl) can be modified to obtain different chitosan derivatives, and provide opportunities for chemical modification to impart useful physicochemical properties and distinctive biological functions [3]. In addition, the advantage of chitosan over other polysaccharides is that its chemical structure allows specific modifications at the C-2 position without too many difficulties [4]. Chitosan has been investigated widely for its potential in the development of drug delivery systems and pharmaceutical applications [5] and more recently for its forensic applications [6].

In latent fingerprint visualisation it is now accepted that particles adhere to fingermarks due to the mechanical attraction with the oily subcutaneous residues [7]. The factors with influence this interaction are particle size, particle charge, particle shape and relative surface area [7,8] all of which are controlled by processing parameters such as chitosan concentration, pH and ionic strength of the dissolution media, temperature of cross-linking, stirring rate, *etc* [9].

Various techniques have been developed to prepare chitosan micro/nanoparticles, such as ionic gelation, emulsion droplet, spray drying, coacervation and self-assembly chemical modification [10]. Among those methods, the ionic gelation method (also known as ionotropic gelation) with the non-toxic multivalent polyanion tripolyphosphate (TPP) is the most widely used approach to physical cross-linking. Ionic cross-linking can occur inside the network *via* interactions between the negative charges of the cross-linker such as TPP and the positively charged amino groups of chitosan molecules [11–14]. This method is advantageous as the reaction is simple and the conditions are relatively mild and do not require the use of organic solvents or high temperatures [1,15]. Other advantages from the point of view of drug delivery and latent fingerprint enhancement are that particle size and (positive) charge can be easily controlled and microparticle formulations have previously

* Corresponding author.
E-mail address: g.morris@hud.ac.uk (G.A. Morris).

http://dx.doi.org/10.1016/j.ijbiomac.2016.11.092
0141-8130/

Table 1
Acetate buffers of varying ionic strength and pH. Buffers AB-1 to AB-9 were used to create generate model equations and buffer AB-10 to AB-13 were used in model validation.

Acetate buffer (AB)	pH	Ionic strength (IS)
AB-1	3.3	0.1 M
AB-2	3.3	0.3 M
AB-3	3.3	0.5 M
AB-4	4.3	0.1 M
AB-5	4.3	0.3 M
AB-6	4.3	0.5 M
AB-7	5.3	0.1 M
AB-8	5.3	0.3 M
AB-9	5.3	0.5 M
AB-10	3.8	0.2 M
AB-11	3.8	0.4 M
AB-12	4.8	0.2 M
AB-13	4.8	0.4 M

demonstrated the ability to associate with peptides, proteins [16] and with subcutaneous secretions in fingerprints [6].

Knowledge of viscosity, zeta potential, particle size and shape has an influence on potential applications of chitosan-TPP microparticles in drug delivery [9] or in forensic applications [6]. It is therefore the purpose of the present study is to investigate the systematic manipulation of three independent processing parameters (pH, ionic strength and CS:TPP ratio) on three important physico-chemical properties (relative viscosity, zeta potential and particle size) during the preparation of chitosan-TPP (CS-TPP) microparticles. This will then enable the use of mathematical models obtained to predict the relative viscosity, zeta potential (net surface charge) and particle size under different conditions to obtain predicable and programmable microparticle properties in relation to, for example, latent fingerprint enhancement, drug release kinetics or mucoadhesion.

2. Materials and methods

2.1. Materials

Chitosan of medium molecular weight (Mη ~295,000 g/mol) was obtained from Sigma–Aldrich (Gillingham, UK) and reported to have an average degree of deacetylation (DD) of ~75–85%. Glacial acetic acid, sodium acetate trihydrate and tripolyphosphate (TPP) sodium salt were obtained from Sigma–Aldrich (Gillingham, UK) and red food colouring was from Silver Spoon (Peterborough, UK). All materials were used without any further purification.

2.2. Sample preparation

Nine different acetate buffers (AB) including AB-1, AB-2, AB-3, AB-4, AB-5, AB-6, AB-7, AB-8, and AB-9 were prepared (Table 1) in order to investigate the effect of three independent variables: pH value, ionic strength and volumetric ratio of chitosan to TPP on the physico-chemical properties of CS-TPP microparticles.

2.2.1. Preparation of chitosan and TPP samples with different ionic strengths and pH value (Acetate buffers AB-1 to AB-9)

Nine different chitosan solutions were prepared by dissolving 2 g of chitosan powder in 1 L of acetate buffers (AB-1 to AB-9) to prepare chitosan solutions (2.0 mg/mL). The chitosan solutions were stirred overnight at room temperature using a magnetic stirrer. The TPP powder (1.680 g) was dissolved in 2 L of the acetate buffers (AB) to prepare nine samples of TPP solution (0.84 mg/mL) [17,18].

2.2.2. Preparation of CS:TPP microparticles

To prepare the CS:TPP microparticles, an appropriate volume of the TPP solution was added drop wise to the appropriate volume of the chitosan solution make seven ratios of CS:TPP microparticles (6:1, 4:1, 2:1, 1:1, 1:2, 1:2, 1:4 and 1:6), and the samples were then stirred at 600 rpm for 60 min at room temperature. The resultant microparticles spontaneously formed due to the ionic crosslinking of chitosan by sodium tripolyphosphate. Then 30 drops (~2 mL) of red dye added to all ratios to make the particles clearly visible and more amenable in latent fingerprint visualisation. The resultant microparticles were left standing overnight at room temperature before being subjected to further analysis.

2.2.3. Model validation (prediction method)

Chitosan solutions were prepared by dissolving 2 mg/mL of polymer in a further four different acetate buffers (AB-10, AB-11, AB-12 and AB-13) (Table 1) and TPP solutions were prepared by dissolving TPP at a concentration of 0.84 mg/mL in the same acetate buffers (AB-10, AB-11, AB-12 and AB-13). The resultant solutions were as in Section 2.2.2 to give CS:TPP volume ratios (v/v) of 6:1, 4:1, 2:1, 1:1, 1:2, 1:4 and 1:6 respectively.

2.3. Characterisation of chitosan microparticles

2.3.1. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of chitosan, TPP and chitosan microparticles were recorded using a Fourier transform infrared spectrophotometer (Thermo Nicolet 380 FT-IR spectrometer, Thermo Electron Corporation), operating from 4000 to 500 cm^{-1}.

2.3.2. Powder X-ray diffraction (XRD) study

A crystallinity study was carried out by comparing XRD spectrum of microparticles using Bruker AXS diffractometer (D2 PHASER) with Cu Kα radiation to characterise chitosan, TPP and CS/TPP microparticles. The data was recorded at 2θ range of 5°–100° at a scanning rate of 4°/min.

2.3.3. Determination of relative viscosities

All rheological measurements (solutions and reference solvents) were performed using a Bohlin Gemini HR Nano Rheometer (Malvern Instruments, Worcester-shire, UK) using 1 mm gap and 55 mm parallel plate geometry at a constant shear rate of 500 s^{-1} under precise temperature control (25.0 ± 0.1 °C). All measurements were performed in triplicate.

$$\eta_{rel} = \left(\frac{\eta}{\eta_0}\right) \quad (1)$$

where η is the average (n = 3) viscosity of the CS:TPP microparticles and, η_o is the viscosity for the appropriate acetate buffer [19].

2.3.4. Determination of zeta potential

Zeta potential was measured for each volume ratio using a Malvern Zetasizer NANO-Z (Malvern Instruments Limited, Malvern, UK). All Measurements were performed in the appropriate buffers in triplicate by using a folded capillary cell at 25.0 ± 0.1 °C and refractive index of the CS:TPP microparticles was set at 1.6–1.8 [20] and no significant effect of refractive index was identified. Each data value is an average of three measurements with a refractive index of 1.8.

2.3.5. Determination of particle size

The particle size distributions of the resultant chitosan particles were measured directly by a dynamic light scattering using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK).The microparticles were dispersed in deionized water. Refractive index of particles and dispersion medium (water) was set to 1.8