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A rational approach towards the design of chitosan-based nanoparticles obtained by ionotropic gelation

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ABSTRACT

Chitosan is a linear aminopolysaccharide that has been widely used for the formation of chitosan-based nanoparticles by ionic gelation with sodium tripolyphosphate (TPP). Often, the experimental design used to obtain these systems does not take into consideration important variables, such as the degree of acetylation (DA) and the molecular weight (M_w) of chitosan. In this work, we studied the formation of chitosan-TPP nanoparticles with chitosan samples of varying DA and M_w (DA0 ~ 0–47% and M_w ~ 2.5–282 kDa). We addressed the influence the degree of space occupancy and the degree of crosslinking on the physical properties of chitosan-TPP nanoparticles. Nanoparticles that comprised chitosan of DA ~ 0–21.7% behaved differently than those made of chitosan of DA ~ 34.7–47%. We attributed these differences to the polymer conformation and chain flexibility of the distinct chitosans in solution. Moreover, chitosan of high M_w were found to have a stronger preference for incorporating into the formed nanoparticles than do low-M_w ones, as determined by SEC-HPLC. These results open new perspectives to understand the formation of chitosan nanoparticles by the ionic gelation technique.

1. Introduction

Chitosan is a linear aminopolysaccharide consisting of 2-deoxy-D-glucosamine (GlcN) and 2-deoxy-*N*-acetyl-D-glucosamine (GlcNAc) units (Scheme 1) [1,2]. It is derived from the deacetylation of chitin and is one of the few naturally-occurring cationic polymers [3]. By contrast with chitin, chitosan dissolves in dilute acidic solution upon protonation of the $-NH_2$ groups at GlcN residues [4]. Chitosan structure can vary in terms of its degree of polymerization (DP), % molar degree of acetylation (DA) and pattern of distribution of GlcN and GlcNAc residues (PA) [1,5]. At present, commercially available chitosan, produced by chemical processes, commonly has a random PA and only limited information is available about the role of chitosan's PA, most of it related to small oligomers [6]. For longer chains (DP > 20), the ordinary parameters to take into consideration are the molecular weight (M_w) and the charge density (related to the DA). Chitosan has received increasing attention in the last two decades, particularly in the biological fields, mostly due to the distinctive physicochemical and bioactive properties that single it out among other natural and synthetic polymers [7–9]. In particular, the ability to interact with different compounds and its limited solubility make chitosan an attractive excipient for galenic formulations [6,10,11].

Chitosan-based nanoparticles are used as a suitable technological platform for several applications including diagnostics, drug and gene delivery and plant protection [12–14]. Different types of nanoparticles can be produced depending on the processing protocols. Ionotropic gelation is among the most widespread techniques for the preparation of chitosan nanoparticles [15–25]. It is a bottom-up approach technique based on a sol–gel transition of chitosan in the presence of polyanions with high negative charge density, such as citrate, sulfate and phosphate ions. Among the advantages of this technique are the aqueous and mild conditions of preparation, the absence of organic solvents and very low requirements of energy [18] as well as the high compatibility with hydrophilic molecules [19]. However, the preparation of chitosan nanoparticles by ionic gelation faces some reproducibility issues. It is not uncommon that nanoparticles prepared by different batches of chitosan show different physical properties and biological responses. One reason for this behavior is the poor characterization of chitosan samples that will consequently influence the reproducibility of the results. Another issue is the poor experimental design and inadequate

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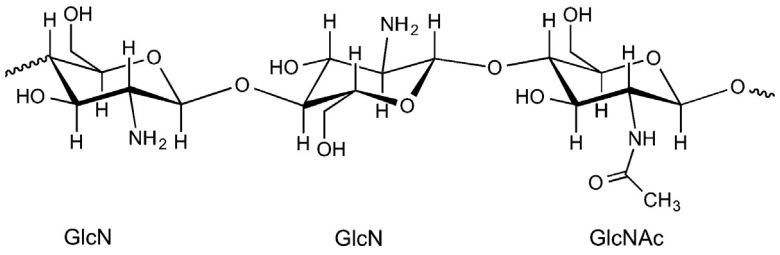

Scheme 1. Schematic representation of a chitosan chain comprising β(1 → 4)-linked 2-acetamide-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN) monosaccharide residues.

selection of the involved variables. Among the variables that have been studied are (i) concentration of chitosan and crosslinker in initial solutions; (ii) the volume ratio between the chitosan solution and the crosslinker solution; (iii) the mass ratio between chitosan and crosslinker; (iv) pH, (v) ionic strength, and (vi) temperature [18,20–23]. Previous studies have also attempted to describe the influence of the M_w of chitosan on the formation of nanoparticles [24]. Although many studies have offered convincing evidence of the importance of optimization of the involved variables, some fundamental aspects have been neglected. For instance, chitosans of different DA lead to assessing different effective molar ratio of chitosan:crosslinker while fixing the mass ratio, and hence, to particles of different physical properties. The optimal amount of crosslinker is often determined by a process of trial and error. In an accompanying paper, we have aimed to study the influence of chitosan's DA and M_w on the size and stability in biological media of chitosan-TPP nanoparticles [25]. We have shown that the DA and *M*w of the comprising chitosan influences directly the size, zeta potential and colloidal stability in biological media of the nanoparticles. The present work, therefore, aims to deepen our understanding of the fundamental parameters and mechanisms that are at play and influence the formation of chitosan nanoparticles by ionotropic gelation. To this end, we have investigated the combined role of the concentration and M_w of chitosan, expressed as the value of the so-called "coil overlap parameter" (given by the product of the concentration and the intrinsic viscosity), on the physical properties and formation of nanoparticles. We also investigated the effect of the degree of crosslinking, given by the molar ratio of chitosan to sodium tripolyphosphate (TPP) for fully characterized chitosan of varying DA. Finally, we studied the influence of the M_w of chitosans of different DA by analyzing the partitioning of the polymer between the nanoparticles and the soluble fraction present in the supernatant after isolating the nanoparticles.

2. Materials and methods

2.1. Materials

The parent chitosan samples (Code 132 Batch No. 17/12/14 and Code 134 Batch No. 25/06/10, here referred to as CS-12HDP and CS-0HDP, respectively; their full characterization is shown in Table 1) were provided by Sascha Mahtani Chitosan PVT Ltd. (Veraval, India). All used reagents were of analytical grade and supplied by Sigma–Aldrich (Germany). Milli-Q water was used throughout. Stock chitosan solutions were filtered with a 5 μm filter (Millipore) before usage.

2.2. Depolymerization of chitosan

Chitosan sample CS-12HDP was dissolved in 5% stoichiometric excess of acetic acid solution at ca. 25 °C and depolymerized using sodium nitrite (forming nitrous acid in situ) as previously reported [27,28]. This enabled to obtain depolymerized chitosans of constant DA (~12%) and varying M_w (samples CS-12 MDP and CS-12 LDP, for details see Table 1).

2.3. N-Acetylation and characterization of chitosan

Chitosan sample CS-0HDP was re-acetylated using acetic anhydride to reach samples with DA of 21.7 (CS-22HDP), 34.7 (CS-35HDP), and 47% (CS-47HDP), as described elsewhere [29] (the full characteristics are given in Table 1). Briefly, chitosan was dissolved in 5% stoichiometric excess acetic acid; clarified by filtration

Table 1
Characteristics of chitosan samples.

	CS-0 HDP	CS-12 HDP	CS-12 MDP	CS-12 LDP	CS- 22 HDP	CS-35 HDP	CS-47 HDP
M_{CS} (g/mol)[a]	161.15	166.04	166.04	166.04	171.26	176.7	181.87
DA (%)[b]	0	12.0	12.0	12.0	21.7	34.7	47.0
M_n (g/mol)[c]	79.869	n.d.	n.d.	n.d.	105.020	97.395	118.220
M_w (g/mol)	99.610[d]	139.200[e]	31.200[e]	2500[e]	200.930[d]	222.500[d]	282.060[d]
I_p[f]	1.25	n.d.	n.d.	n.d.	1.91	2.28	2.39
DP[g]	618	838	187	15	1173	1259	1551
dn/dc[h]	0.1985	0.1895	0.1895	0.1895	0.1844	0.1823	0.1824
[η] (mL/g)[i]	1030	1151	440	163	856	882	792

n.d. = Not determined.
[a] Average molar mass per residue.
[b] Degree of *N*-acetylation as determined by ^{1}H NMR.
[c] Number average molecular weight determined by SEC-HPLC with MALLS-DRI detection.
[d] Weight average molecular weight determined by SEC-HPLC with MALLS-DRI detection.
[e] Viscosimetric molecular weight determined in 0.3 M acetic acid/0.2 M sodium acetate (25 °C) [26].
[f] Polydispersity index ($I_p = M_w/M_n$)
[g] Degree of polymerization.
[h] Differential refractive index.
[i] Intrinsic viscosity determined in aqueous acetic acid (5% stoichiometric excess; 0.085 mM NaCl; 20 °C).