Colloids and Surfaces B: Biointerfaces 115 (2014) 100–108

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Formation and dissolution of chitosan/pyrophosphate nanoparticles: Is the ionic crosslinking of chitosan reversible?

Yuhang Cai[a], Yakov Lapitsky[a,b,*]

[a] *Dept. of Chemical and Environmental Engineering, University of Toledo, Toledo, OH 43606, USA*
[b] *School of Green Chemistry and Engineering, University of Toledo, Toledo, OH 43606, USA*

ARTICLE INFO

Article history:
Received 31 July 2013
Received in revised form
14 November 2013
Accepted 15 November 2013
Available online 24 November 2013

Keywords:
Chitosan
Pyrophosphate
Ionic crosslinking
Nanoparticles
Hysteresis

ABSTRACT

Ionically crosslinked chitosan particles with submicron dimensions attract widespread interest as materials for controlled release. To this end, we have examined the formation and dissolution of nanoparticles prepared by crosslinking chitosan with pyrophosphate (PPi). The formation of these particles required a critical PPi concentration (which increased with the chitosan concentration), and their z-average hydrodynamic diameters could be predictably tuned from roughly 60 to 220 nm by varying the concentration of the parent chitosan solutions. Unlike the nanoparticles crosslinked with the commonly used tripolyphosphate (TPP), which coagulated and precipitated when TPP was in excess, the chitosan/PPi nanoparticles remained colloidally stable even at high PPi concentrations. Moreover, the analysis of their dissolution revealed hysteresis in the particle formation/dissolution cycle, where portions of the crosslinked chitosan/PPi complexes remained stably intact at PPi concentrations below those required for their formation. This irreversible behavior was surmised to reflect the cooperativity of chitosan/PPi binding and was qualitatively modeled using the Bragg–Williams theory.

1. Introduction

Ionically crosslinked chitosan particles with submicron dimensions are widely studied as materials for the delivery of drugs, genes and food additives [1–4]. They are typically prepared by mixing multivalent anions, such as tripolyphosphate (TPP), with dilute chitosan solutions (see Figs. 1a and 1b), which leads to spontaneous micro- and nanoparticle formation. These colloidal carriers provide multiple advantages, such as forming under very mild conditions [5], being biocompatible and mucoadhesive [5–8], enhancing drug penetration across epithelial membranes [9,10], and stabilizing proteins against denaturation [11].

Despite the numerous studies exploring their preparation and use [1–6,9,10,12,13], work with these materials has mostly been limited to particles formed using TPP as the crosslinker. While recently there has been some interest in submicron particles prepared using other ionic crosslinkers (e.g., pyrophosphate (PPi) (Fig. 1c) [14,15] and sulfate [16]), factors controlling their formation, structure and stability have not been studied in detail. Therefore, although these alternative crosslinkers could yield a broader range of desirable particle properties, there is a dearth of guidelines for their use.

Furthermore, while much attention has been devoted to studying particle formation and size distributions [1,3,12,13,17], little has been done to investigate the dissociation of ionically crosslinked chitosan particles. When ionically crosslinked particles are placed into an excess of crosslinker-free solution (i.e., during their application) it is probable that the ionic crosslinker is leached from the particles, causing the particles to dissolve. Yet, although several studies have examined the shelf life of chitosan/TPP particles [18–20], and it is known that chitosan crosslinked with TPP or PPi dissolves in the limit of low pH (e.g., pH 1, where the crosslinking anions become protonated) [21,22], the dissolution of ionically crosslinked chitosan through simple crosslinker leaching (achieved without pH reduction) remains virtually unexplored. Indeed, the only such work known to us was a protein uptake/release study where the structure of protein-laden chitosan/TPP particles became less dense during in vitro protein release experiments [23]. Due to the presence of protein, however, the role of crosslinker leaching in these structural changes (versus that of protein elution) remains unclear.

To begin addressing these issues, the present study had two objectives: (1) to provide systematic guidelines for the preparation of chitosan/PPi nanoparticles which, as shown herein, behave dissimilarly from those prepared using TPP; and (2) to investigate the dissolution of ionically crosslinked chitosan particles. This

---

* Corresponding author at: Department of Chemical and Environmental Engineering, University of Toledo, Toledo, OH 43606, USA. Tel.: +1 419 530 8254.
E-mail address: yakov.lapitsky@utoledo.edu (Y. Lapitsky).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.11.032

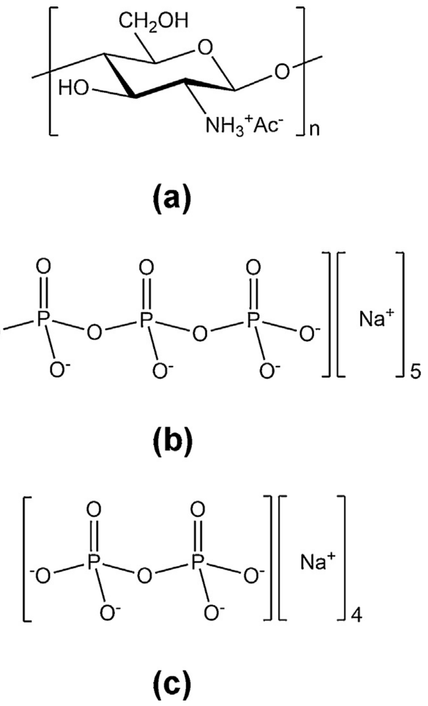

Fig. 1. The molecular structures of (a) chitosan (b) TPP (c) PPi.

second objective was pursued using PPi instead of the more-common TPP because chitosan/PPi binding was much weaker than chitosan/TPP binding [14]. In other words, because the very strong chitosan/TPP binding made TPP elution challenging to achieve [22], the PPi-based particles provided a more-tractable experimental system. Based on these considerations, this study consisted of three parts. First, dynamic and electrophoretic light scattering, scanning transmission electron microscopy (STEM), stopped-flow turbidimetry and viscometry were used to investigate the compositional parameters affecting the formation, structure and colloidal stability of chitosan/PPi nanoparticles. Second, light scattering was used to investigate particle dissolution that occurred when PPi was leached from the complex. Third, the irreversible formation/dissolution behavior revealed by these experiments was qualitatively modeled using the Bragg–Williams theory.

2. Materials and methods

Low molecular weight chitosan (nominal MW = 50–190 kDa), sodium pyrophosphate (Na_2PO_4) and acetic acid were purchased from Sigma–Aldrich (St. Louis, MO) and used as received. The degree of chitosan deacetylation was 90% [24]. All experiments were performed using 18.2 MΩ m Millipore Direct-Q 3 deionized water at pH 4.0 (using acetic acid to adjust the pH) and, unless otherwise noted, at room temperature.

To prepare the particles, 10 mL of 0.01–0.20 wt% chitosan solution (0.55–11 mM in its cationic monomer units) were stirred at 800 rpm with a cylindrical magnetic stir bar (12 mm × 4 mm) and titrated with 0.37 wt% (14 mM) PPi solution. The PPi solution was added in 50–200-μL increments, at a rate of two drops per second. After each PPi addition the mixtures were equilibrated for 10 min, whereupon light scattering and ζ-potential analysis was performed to detect particle formation and characterize the evolution in particle size, charge and colloidal stability. These measurements were performed using a Zetasizer Nano ZS (Malvern, UK) dynamic and electrophoretic light scattering instrument, wherein the light scattering intensities were reported as derived count rates (i.e., scattering intensities that would be obtained if each measurement was conducted at full laser power [25]) to facilitate the comparison between strongly and weakly scattering samples.

The dynamic light scattering (DLS) particle size measurements were confirmed by STEM, using a Hitachi HD-2300 microscope. Here, the particle dispersions prepared from 0.03 and 0.10 wt% chitosan solutions (titrated to 2.5 and 3.6 mM PPi, respectively) were deposited onto carbon grids (carbon type A; Ted Pella, Redding, CA). The samples were then dried for 10 min and imaged using a 200 kV acceleration voltage. Further characterization of the particle dispersions was performed via capillary viscometry (at 25 °C), using a Cannon-Ubbelohde Dilution viscometer (Size 75; Cannon Instrument Co., State College, PA). Additionally, the effect of PPi concentration on the nanoparticle formation kinetics was probed by stopped-flow turbidimetry, using a DM 45 K stopped-flow spectrophotometer (Olis Inc., Bogart, GA). Here, 0.10 wt% (5.5 mM) chitosan and 0.18–0.55 wt% (6.9–21 mM) PPi solutions were rapidly mixed in the stopped-flow sample cell in a 5:1 chitosan:PPi solution volume ratio. The formation kinetics were then inferred from the evolution in turbidity (where the formation of particles yielded more scattered light).

To examine particle dissolution (and the reversibility of the chitosan/PPi particle formation), a two-step experiment was performed. In the first, “forward titration” step, 10-mL samples of 0.10 wt% (5.5 mM) chitosan solution were titrated with 14 mM PPi solution to final PPi concentrations ranging between 0.2 and 2.0 mM. The titrations were performed as series of 200-μL additions (conducted at a rate of 2 drops per second) separated by 1-min time intervals. The light scattering from these samples was then tracked for 29 d. Each mixture was stirred for the first day after preparation, and then stored at rest.

In the second, “backward dilution” step, samples containing 2.0 mM PPi were first prepared as described in the forward titration step. After stirring for 1 d, however, these samples were diluted with 0.10 wt% chitosan solution to final PPi concentrations ranging between 0.2 and 1.7 mM, so that the “forward titration” and “backward dilution” samples had matching chitosan and PPi concentrations. Because the particles dissolved at low PPi concentrations, this allowed the determination of whether the particle formation/dissolution cycle was thermodynamically reversible. As in the forward titration, the diluted dispersions were stirred for the first day after preparation, and then stored at rest. To monitor their dissolution, the evolution in their light scattering intensity was tracked for 28 d, and characterized at the same times as the “forward titration” samples (so that the time elapsed since the initial PPi addition to chitosan was the same for both the “forward titration” and “backward dilution” samples). The sample analysis was limited to the 4 weeks after dilution, to reduce the effects of chitosan and PPi hydrolysis and, to ensure reproducibility, this experiment was repeated thrice.

3. Results and discussion

3.1. Formation and characterization of chitosan/PPi particles

3.1.1. Formation of chitosan/PPi particles

The formation of chitosan/PPi particles was determined from the changes in light scattering intensity during the titration of PPi into chitosan solutions. At low PPi concentrations (where no particles formed) the light scattering intensity remained similar to that of PPi-free chitosan solutions. At the onset of particle formation, however, the scattering intensity began to suddenly increase (see Fig. 2a). Finally, at even higher PPi concentrations, where the particle formation process ended, the light scattering intensity plateaued. These trends occurred at each chitosan concentration and were consistent with those recently reported by Huang and