Carbohydrate Polymers 87 (2012) 1670–1677

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Rheological properties of chitosan–tripolyphosphate complexes: From suspensions to microgels

Ji Li, Qingrong Huang*

Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA

ARTICLE INFO

Article history:
Received 22 February 2011
Received in revised form 4 September 2011
Accepted 26 September 2011
Available online 1 October 2011

Keywords:
Chitosan (CS)
Sodium tripolyphosphate (TPP)
Rheological property
Particle packing

ABSTRACT

Complex fluids formed by crosslinking of chitosan (CS, 330 kDa) with sodium tripolyphosphate (TPP) have been studied by dynamic light scattering (DLS), atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), and rheology. The effects of chitosan/TPP ratios, initial chitosan or TPP concentrations, and ultrasonication time on the chitosan–TPP complex formation have been investigated. It was found that the optimum condition for CS–TPP nanoparticle formation occurred at CS/TPP mass ratio of 3.75 and with 9 min sonication treatment (energy output 3.75 W/mL). At the same initial chitosan concentration, small particle sizes (i.e., particle size < 300 nm) resulted in the formation of CS–TPP nanoparticle suspensions, which showed a lower viscosity than pure chitosan solutions, and their viscosities increased as the CS–TPP nanoparticles sizes increased. Centrifugation of CS–TPP particles of larger particle sizes (i.e., 360–870 nm) at 11,000 × *g* caused the formation of CS–TPP microgels. Dynamic rheological studies indicated that both storage modulus (G') and loss modulus (G'') increased with particle sizes. During centrifugation processing, strong centrifugal force surmounted the electrostatic repulsion between CS–TPP particles and caused particles to stick with each other to form CS–TPP microgels. The water contents of microgels negligibly depended on particle size, suggesting that the free volumes of microgels were not affected by particle size, therefore supporting our pseudo-hard sphere assumption for CS–TPP nanoparticles.

1. Introduction

Chitosan (CS), the second most abundant biopolymer in nature next to cellulose, is one of the very few positively charged natural biopolymers existing in the world. It is derived from the exoskeleton of shrimps and other crustaceans, and has a linear structure composed of glucosamine unit and N-deacetylated glucosamine unit, also known as 2-amino-2-deoxy-(1 → 4)-β-D-glucopyranan. Chitosan has received broad attention from researchers of different backgrounds due to its unique structure and natural abundance (Yi et al., 2005). Previous literatures show that chitosan has been used to form complex coacervates (Espinosa-Andrews, Baez-Gonzalez, Cruz-Sosa, & Vernon-Carter, 2007), biocomposites (Luo et al., 2008), bio-carbon nanotubes (Zhang, Smith, & Gorski, 2004), and scaffolds for tissue engineering (Skotak, Leonov, Larsen, Noriega, & Subramanian, 2008). Other applications of chitosan include drug delivery systems, nanofibers, biosensors, and edible films (Han, Shan, Xue, & Cosnier, 2007; Zhang, Mardyani, Chan, & Kumacheva, 2006; Zhang, Su, Ramakrishna, & Lim, 2008). Among the above research areas, chitosan-based delivery system is one of the most important applications due to its biodegradability, biocompatibility, bioadhesion and non-toxicity (Janes, Calvo, & Alonso, 2001; Pillai & Panchagnula, 2001; Sogias, Williams, & Khutoryanskiy, 2008). Many investigations of chitosan-based delivery systems have been carried out previously. For example, Jang and Lee (2008) succeeded in improving the heat stability of L-ascorbic acid during processing by utilizing chitosan–TPP nanoparticles (Jang & Lee, 2008). Richardson, Kolbe, and Duncan (1999) conjugated chitosan to DNA backbone for protecting DNA from endonuclease degradation and promoting DNA's cell targeting (Richardson et al., 1999). Wu, Yang, Wang, Hu, and Fu (2005) applied CS–TPP nanoparticles for loading the drug ammonium glycyrrhizinate. The release profile of their CS–TPP nanoparticles followed the rule of first burst release and then steady release, suggesting that CS–TPP nanoparticle was a suitable oral delivery agent (Wu et al., 2005).

In order to meet different demands, distinct methods were used to produce chitosan nanoparticles. Chemical modification provides us with series of methods for producing stable chitosan nanoparticles. For instance, amphiphilic micellar structure of linolenic acid-modified chitosan could be immobilized with trypsin by using glutaraldehyde as the crosslinker, which greatly improved trypsin's thermal stability and enzymatic activity (Liu, Desai, Chen, & Park, 2005). Other researchers functionalized chitosan with multiple functional groups, such as octyl, sulfate and

* Corresponding author. Tel.: +1 732 932 7193; fax: +1 732 932 6776.
E-mail address: qhuang@aesop.rutgers.edu (Q. Huang).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.09.074

polyethylene glycol monomethyl ether (mPEG) groups to target both polymeric micelle structure and brain-targeting function, and the resulted chitosan nanoparticles could improve the water solubility of hydrophobic drug paclitaxel by 4000 times (Yao, Zhang, Ping, and Yu, 2007). Very recently, a novel chitosan-based amphiphile, octanoylchitosan–polyethylene glycol monomethyl ether (acylChitoMPEG), has been synthesized using both hydrophobic octanoyl and hydrophilic polyethylene glycol monomethyl ether (MPEG) substitutions (Huang, Yu, Guo, & Huang, 2010). The synthesized acylChitoMPEG exhibited good solubility in either aqueous solution or common organic solvents such as ethanol, acetone, and $CHCl_3$. Cytotoxicity results showed that acylChitoMPEG exhibited negligible cytotoxicity even at the concentration as high as 1 mg/mL (Huang et al., 2010).

In addition to chemical synthesis, physical methods were also used to create chitosan complexes or nanoparticles with milder processing conditions. Since chitosan has hydroxyl and amino groups on the backbone, chitosan can interact with other negatively charged hydrocolloids or small molecular weight compounds to form complexes. These complexes could potentially be used for mouth-feel improvement in food industry (Carvalho et al., 2006) and drug delivery in pharmaceutical industry (Weinbreck, Tromp, & de Kruif, 2004). Gum arabic is a thickening agent commonly used in food product development, such as flavor encapsulation. Espinosa-Andrews et al. (2007) investigated the interactions between gum arabic and chitosan by examining the influence of gum arabic/chitosan ratio, total polymer concentration, pH and ionic strength upon the electrostatic complexes formation. Their turbidity and electrophoretic mobility results showed that the optimized gum arabic/chitosan mass ratio was 5 for coacervate formation. The maximized gum arabic–chitosan interaction could be obtained within the pH range between 3.5 and 5 (Espinosa-Andrews et al., 2007). Another negatively charged compound worth noting is sodium tripolyphosphate (TPP), a small molecular weight crosslinker carrying five negative charges in each molecule. TPP has been approved as a GRAS ("generally recognized as safe") reagent by FDA. Chitosan (CS) and TPP can form nanoparticles through electrostatic interaction, which has previously been investigated for different delivery applications (Gan & Wang, 2007; Hu et al., 2008; Jang & Lee, 2008; Ko, Park, Hwang, Park, & Lee, 2002; Wu et al., 2005). One interesting formulation among them is CS–TPP nanoparticles developed through an O/W emulsion route for entrapping hydrophobic felodipine (Ko et al., 2002). After felodipine was entrapped into CS–TPP nanoparticles, the control release of felodipine could be achieved by tuning pH, initial concentration, and molecular weight during nanoparticles preparation.

Previous studies suggest that CS–TPP nanoparticles are very useful carriers for drug and nutraceutical delivery. It is known that the CS–TPP particles were formed mainly through the electrostatic interaction between positively charged chitosan and negatively charged TPP molecules. However, how the CS–TPP particle sizes affect their packing, as well as the rheological properties of the resulted complex fluids (either chitosan–TPP particle suspensions or microgels) have been scarcely reported. In this paper, chitosan particles of different sizes were prepared through the use of TPP and ultrasonication. Depending on particle sizes, either CS–TPP particle suspensions or microgels were obtained after centrifugation at 11,000 × *g*, and their corresponding rheological properties were investigated by both static and dynamic rheological measurements. The static rheological technique measured the apparent viscosity (η) of polymer solution as a function of shear rate, while dynamic frequency test determined the storage modulus (G') and loss modulus (G'') as a function of angular frequency (ω). The correlation between particle sizes and particle packing profiles was also explored through rheological measurements.

2. Materials and methods

2.1. Materials

Chitosan with deacetylation degree (DD) of 98.0% and molecular weight (M_w) of 330 kDa was purchased from Kunpoong Bio. Co., Ltd. (South Korea). Sodium tripolyphosphate (TPP, 85%, technical grade) was purchased from Acros Organics (Morris Plains, NJ). Acetic acid, glacial (ACS grade) was purchased from Fisher Scientific (Fair Lawn, NJ). All of these reagents were used as received. Milli-Q (18.3 MΩ) water was used in all experiments.

2.2. Methods

2.2.1. Preparation of chitosan–sodium tripolyphosphate (CS–TPP) nanoparticles

Different amounts of chitosan (CS, 330 kDa) were dissolved in 2 wt% acetic acid solution to form chitosan solutions with concentrations ranging from 1 mg/mL to 30 mg/mL. Sodium tripolyphosphate (TPP) was dissolved in Milli-Q water to form a 200 mg/mL solution. CS–TPP nanoparticles were formed by dropwise addition of TPP solution into chitosan stock solution at different CS/TPP mass ratios under severe magnetic stirring. The change of solution volume caused by the addition of TPP solution was negligible due to the large CS/TPP volume ratio. After vortexing for 5 min, 40 mL of each CS–TPP particle suspension was processed under ultrasonication (Sonifier Cell Disruptor, Model W-350, Branson Sonic Power Co.) with 3.75 W/mL energy output and the duration varying from 3 to 9 min to obtain chitosan particles with controlled particle sizes. High speed centrifugation at ambient condition was set at 11,000 × *g* for 50 min to separate the microgel and supernatant. The supernatant was then removed, and the remaining microgels were washed with 2 wt% acetic acid buffer for three times prior to the rheological study.

2.2.2. Particle size measurements

Photon correlation spectroscopy (PCS)-based BIC 90 plus particle size analyzer equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corporation, New York, USA) was used to measure hydrodynamic diameters (d) and size distribution of CS–TPP nanoparticles. The light source is a solid state laser operating at the wavelength of 658 nm with 30 mW power, and the signals were detected by a high sensitivity avalanche photodiode detector. All measurements were conducted at 25 ± 1 °C with the detection angle of 90°. CS–TPP nanoparticle suspensions were diluted with buffer until their viscosities were close to that of water (i.e., 0.89 cp at 25 °C). The normalized field–field autocorrelation functions $g(q,t)$ were obtained from the intensity–intensity autocorrelation functions, $G(q,t)$, via the Sigert relation (Stepanek, 1993). Both single stretched exponential fit and Cumulant analysis method were used in our particle size measurements (Wang et al., 2008).

2.2.3. Rheological measurements

Rheological measurements of the CS–TPP nanoparticle suspensions were performed by using ARES Rheometer (Rheometrics Scientific, NJ) with either cone and plate geometry (diameter 50 mm, cone angle 4°) or parallel plate geometry (diameter 25 mm) at ambient temperature (approximately 25 °C). Steady sweep measurements were carried out by applying shear rate from 1 to 1000 s^{-1} with 20 data points per decade. Zero shear viscosities, the viscosities at vanishing shear rates, were determined by extrapolating the Newtonian plateau to zero shear rate. Prior to a dynamic frequency sweep test, dynamic strain sweep test ranging from 0 to 100% was performed at 2 rad/s angular frequency. In this paper, the strain was fixed at 0.5% and the angular frequency ω was ranged