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Strategy to improve the characterization of chitosan by size exclusion chromatography coupled with multi angle laser light scattering

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ABSTRACT

Here we present SEC-MALLS study for chitosan samples with weight average molar mass M_w between 33 and 427 kg/mol on columns packed with 8 μm porous particles. A low injection concentration on the order of 0.1–0.2 mg/mL must be used to avoid overloading of the SEC columns, due to the extended coil conformation of cationic chitosan in the dilute acid buffer as compared to neutral polymers. Additionally, SEC must be performed at an eluent flow rate no more than 0.5 mL/min for high molar mass chitosan samples. At flow rates of 1.0 and 1.5 mL/min, the elution of the largest chitosan species is delayed, which leads to a distortion of the molar mass distribution towards lower molar mass region. Such an abnormal behavior is due to a chromatographic mode transition from SEC to slalom chromatography, originated from the coil–stretch transition of chitosan chains in elongational flow through the packed columns.

1. Introduction

Chitosan is a linear polymer usually produced from partially or fully de-*N*-acetylation of chitin. As an important polysaccharide, chitosan attracts considerable attention due to its multiple biofunctionality, biodegradability and biocompatibility (Bhattarai, Gunn, & Zhang, 2010; Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Roy, Mao, Huang, & Leong, 1999; Shin et al., 2017). It remains the subject of intensive research concerning its molecular characteristics in aqueous solution by various techniques such as viscometry, laser light scattering, analytical ultracentrifugation, size exclusion chromatography (SEC), and field flow fractionation (FFF), etc (Almutairi et al., 2015; Augsten & Mader, 2008; Schatz, Viton, Delair, Pichot, & Domard, 2003; Wang, Bo, Li, & Qin, 1991; Wu, Zhou, & Wang, 1995). The coupling of a fractionation technique, such as FFF or SEC, to static light scattering is especially promising to determine the molar mass and its distribution, as well as chain conformation of polymers in a single measurement without the need to prepare and study narrow distributed samples of different molar mass individually. The experiments on size exclusion chromatography coupled with multi angle static laser light scattering (SEC-MALLS) of chitosan are distinct in conditions as compared to those for the analysis of neutral polymer due to the cationic polyelectrolyte characteristics of chitosan. Usually the mobile phase for SEC analysis of chitosan contains a mildly acidic solution to dissolve chitosan and certain amount of salt to screen the intramolecular electrostatic interaction. The SEC separation of chitosan is typically achieved on columns packed with 6–20 μm hydrophilic polymer particles containing pores of different sizes. Strategies to improve the characterization of chitosan by SEC-MALLS have been proposed in the literature (Brugnerotto, Desbrieres, Roberts, & Rinaudo, 2001; Christensen, Vold, & Varum, 2008; Jiang et al., 2006; Nguyen, Winnik, & Buschmann, 2009; Nguyen, Hisiger, Jolicoeur, Winnik, & Buschmann, 2009; Rinaudo, Milas, & Dung, 1993; Weinhold et al., 2009; Yanagisawa, Kato, Yoshida, & Isogai, 2006), as well as in the testing method ASTM F2602-13 (2013). The typical flow rate of the mobile phase ranged from 0.4 to 1.0 mL/min, and the injection concentration of chitosan solution is on the order of 1 mg/mL, which depends on the molar mass of chitosan. These experimental conditions must be, however, checked for individual chitosan sample in the employed SEC-MALLS system before accurate results can be obtained.

In the present study, we revisit the molar mass determination of chitosan by SEC-MALLS measurements on a series of chitosan samples with different molar mass. The effects of injected polymer concentration and eluent flow rate are studied. The conformation of chitosan in the solution is deduced and compared with the wormlike chain model.

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Table 1
Molecular characteristics of chitosan samples.

Sample	*D.D.* [a] (%)	M_w [b] (kg/mol)	M_w [c] (kg/mol)	M_n [c] (kg/mol)	M_w/M_n [c]
CS 95-5	97.4	25	32.7	11.4	2.86
CS 95-10	95.9	50	71.1	18.6	3.83
CS 95-20	95.4	90	60.5	33.3	1.82
CS 95-50	97.4	150	99.4	48.0	2.07
CS 95-100	97.0	220	127.1	61.7	2.06
CS 95-200	95.2	300	197.2	83.1	2.37
CS 95-500	95.3	350	276.3	98.8	2.80
CS 95-1000	94.9	400	317.1	113.1	2.80
CS 95-2000	94.5	450	367.6	131.0	2.81
CS 95-3000	93.5	600	426.6	155.7	2.74

[a] Obtained from ^{1}H NMR on a Bruker 400 MHz spectrometer.
[b] Estimated from viscosity as given by HMC+.
[c] Determined by SEC-MALLS in this study.

2. Materials and methods

2.1. Materials

Acetic acid (HAc), ammonium acetate (NH_4Ac) and sodium chloride (NaCl) were obtained from Sigma-Aldrich. Toluene was purchased from J. T. Baker. All other regents were of analytical grade. All solutions were prepared using ultrapure water from Sartorious water purification system with a resistivity of 18.2 MΩ cm.

Ten commercial chitosan samples of different molar mass, namely CS 95-5, 95-10, 95-20, 95-50, 95-100, 95-200, 95-500, 95-1000, 95-2000, 95-3000, were purchased from Heppe Medical Chitosan GmbH (HMC+, Halle, Germany). The degree of deacetylation (D.D.) values of these samples are about 95% (Table 1), as determined from their ^{1}H NMR spectra obtained on a Bruker 400 MHz spectrometer at 70 °C, with 2 wt% CD_3COOD/D_2O as a solvent (Hirai, Odani, & Nakajima, 1991; Kasaai, 2010). The number after "-" represents the solution viscosity (mPa.s) of 1% chitosan in 1% HAc aqueous solution at 20 °C. Table 1 also list the molar mass data of these chitosan samples as given by the supplier and those determined by SEC-MALLS in this study, and the weight average molar mass M_w is between 33 and 427 kg/mol. These samples were kept in dark, and were desiccated in vacuum at 40 °C to a constant weight before use.

2.2. SEC-MALLS

An Agilent 1260 size exclusion chromatography coupled with a DAWN HELEOS-II multi angle laser light scattering (Santa Barbara, CA) was used in this study. The eluent was a buffer containing 265 mM HAc and 200 mM NH_4Ac (pH4.5) and was filtered with a 0.22 μm pore size membrane before use. The eluent flow rates used ranged from 0.1 to 1.5 mL/min. Two PL aquagel−OH 8 μm Mixed-H columns (300 × 7.5 mm) from Agilent were used for the separation. These columns are packed with particles of different pore sizes, and cover a broad molar mass range of 6000–10,000,000 g/mol for poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO) with a linear calibration curve.

Sample solutions were typically prepared at concentrations of 0.25 mg/mL for CS 95-5, 95-10, 95-20, 95-50, 95-100 and 0.125 mg/mL for CS 95-200, 95-500, 95-1000, 95-2000, 95-3000 in the buffer. The polymer solutions were filtered through a 0.45 μm hydrophilic PES membrane (Agilent) before injection via an autosampler with an injection volume of 200 μL.

The MALLS detector, with 16 scattering angles between 13.0° and 157.7°, was positioned before the Wyatt Optilab T-Rex (Santa Barbara, CA) refractive index detector, both operating at a wavelength of 658 nm. The MALLS detector was calibrated with toluene and normalized by multiple injection of a narrow PEG standard with M_w = 15,120. The refractive index detector was calibrated with NaCl aqueous solutions of known concentrations.

The refractive index increment (dn/dc) of chitosan in the eluent was determined by the calibrated Optilab T-Rex. A value of 0.193 mL/g for chitosan in the 265 mM HAc/200 mM NH_4Ac buffer (pH4.5) was found, which agreed well with the data in the references (Brugnerotto et al., 2001; Nguyen, Winnik et al., 2009; Nguyen, Hisiger et al., 2009; Wang et al., 1991; Wu et al., 1995).

Wyatt Astra software was used for data acquisition and analysis. Of the four fit methods for polymer analysis embedded in the Astra software, namely Debye, Zimm, Berry, and Random coil fit method, the Berry method with a linear fit was used for low molar mass polymers, CS 95-5 to 95-100. However, for high molar mass chitosan samples, CS 95-200 to 95-3000, the Random coil method was employed to give the most accurate results (Andersson, Wittgren, & Wahlund, 2003; Liu, Radke, & Pasch, 2005).

3. Results and discussion

3.1. Overloading of chitosan samples in the SEC columns

Fig. 1 shows the chromatograms of CS 95-20, 95-200, 95-3000 at different injection concentrations with a fixed eluent flow rate of 0.5 mL/min. The concentrations of the injected chitosan solutions are 0.25–1.0 mg/mL for CS 95-20 and 0.125–0.50 mg/mL for CS 95-200 and 95-3000, respectively. It can be seen that, for all these samples, both signals from the RI and LS detectors increase with increasing

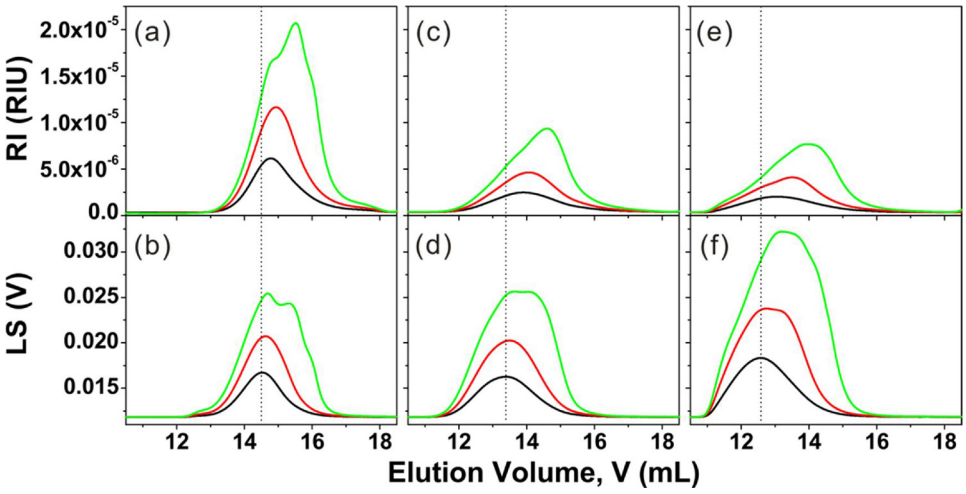

Fig. 1. RI (top panels) and LS responses at 90.0° (bottom panels) for CS 95-20 (a, b), 95-200 (c, d) and 95-3000 (e, f) at different injection concentrations. The black, red and green curves are those obtained at 0.25, 0.50 and 1.00 mg/mL for CS 95-20, and 0.125, 0.25 and 0.50 mg/mL for CS 95-200 and 95-3000, respectively. The vertical dotted lines indicate the peak retention volumes of 14.5 mL for CS 95-20, 13.4 mL for CS 95-200 and 12.6 mL for CS 95-3000, in the low concentration limit, respectively.