Carbohydrate Polymers 102 (2014) 717–726

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Reversible controlled assembly of chitosan and dextran sulfate: A new method for nanoparticle elaboration

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ARTICLE INFO

Article history:
Received 13 September 2013
Received in revised form 25 October 2013
Accepted 31 October 2013
Available online 8 November 2013

Keywords:
Chitosan
Dextran sulfate
Colloids
Reversible assembly
Polyelectrolyte complexes
Nanoparticles

ABSTRACT

Colloidal polyelectrolyte complexes (PECs) were obtained by the controlled assembly of oppositely charged dextran sulfate and chitosan, at room temperature, in water and under moderate stirring. The control over the assembly process was achieved by the slow dialysis of the sodium chloride added to the polyelectrolyte solutions prior to mixing them. This method was carried out at high polymer concentrations of 1.5 wt% and 3 wt%, with screening salt concentrations (SSC) from 2 mol L^{-1} and chitosans of degree of acetylation (*DA*) from 39% and above. The resulting particles featured a size distribution between 350 and 580 nm, a positive surface charge (30–58 mV) and remained stable for 40 days at 37 °C. The reversibility of the controlled assembly was established by adding 2 mol L^{-1} NaCl to the dispersion, the particle solubilized and then re-formed upon dialysis of the salt.

1. Introduction

Polyelectrolyte complexes are formed by mixing aqueous solutions of oppositely charged polymers. This synthesis pathway can be regarded as a green formulation process because it takes place in water, at room temperature, without any organic solvent and with a limited energy input, in comparison with methods involving an emulsification step for instance. Polyelectrolyte complexation leads to a wide variety of materials ranging from soluble complexes (Kabanov, Bronich, Kabanov, Yu, & Eisenberg, 1996; Kabanov & Zezin, 1984), through coacervates, which are complex rich liquid phases (Spruijt, Cohen Stuart, & van der Gucht, 2013), to precipitates (Chollakup, Smitthipong, Eisenbach, & Tirrell, 2010) of various morphologies such as membranes, particles fibers, etc. (Spruijt et al., 2013). The morphologies of the precipitates arise from the control of the course of the assembly via a variety of parameters, like the respective charge density and degree of polymerization of the two counterparts (Gucht, Spruijt, Lemmers, & Cohen Stuart, 2011), the polymer concentration (Schatz, Domard, Viton, Pichot, & Delair, 2004), the positive to negative charge mixing ratio (César et al., 2013), the polymer architecture (Hales & Pochan, 2006), the temperature (Chollakup et al., 2010) and ionic strength of the continuous phase (Dautzenberg, 1997; Kabanov & Zezin, 1984) and, finally, the mode of processing (Mihai, Dragan, Schwarz, & Janke, 2007).

Polyelectrolyte complexes from polysaccharides have a wide potential of application in Life Sciences, e.g. as drug delivery systems (Lankalapalli & Kolapalli, 2009), imaging tools (Hartig, Greene, Dikov, Prokop, & Davidson, 2007) or in tissue engineering (Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012), because they are based on biosourced raw materials and formulated via a green process which, *à priori*, reinforce their safety profile. Among these new materials, colloidal polyeclectrolyte complexes have received great attention as new tools for nanomedicine, using chitosan, a β (1→4) copolymer of glucosamine and *N*-acetyl glucosamine, the only naturally occurring polycation (Hamman, 2010). A great variety of anionic polysaccharides can form polyelectrolyte complexes in the colloidal size domain with chitosan, for example, carboxymethyl cellulose (Douglas & Tabrizian, 2005; Ichikawa, Iwamoto, & Watanabe, 2005), alginates (Douglas & Tabrizian, 2005), carboxymethyl konjac glucomannan (Du et al., 2005), hyaluronan and heparin (Boddohi, Moore, Johnson, & Kipper, 2009) and dextran sulfate (Delair, 2011).

The formation mechanism of chitosan–dextran sulfate colloidal polyelectrolyte complex was largely investigated: pH and ionic strength of the continuous phase, charge mixing ratio, respective molar mass of the polymer counterparts, the mode of addition were scrutinized (Schatz, Domard, et al., 2004; Schatz, Lucas, Viton, Domard, Pichot, & Delair, 2004); the stoichiometry of the complexes was found different to a 1:1 charge neutralization and

Abbreviations: Cc, critical salt concentration; SSC, screening salt concentration; CDS, complex dissociating salt; PSC, particles solid content.

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http://dx.doi.org/10.1016/j.carbpol.2013.10.098

depended on the nature of the polymer used in excess (Drogoz, David, Rochas, Domard, & Delair, 2007); intrinsic parameters of chitosan, i.e. molar mass and degree of acetylation (*DA*, corresponding to the molar fraction of *N*-acetyl groups in the polymer chain) had a strong impact on the colloidal stability of the nanocomplexes (Weber et al., 2010). Interestingly, conversely to synthetic polymers, the chitosan–dextran sulfate assemblies were irreversible, i.e. the complexes would not rearrange after the addition of an excess of one of the counterparts, as a result of the cooperative interactions of hydrogen bonding and hydrophobic interactions (Schatz, Lucas, et al., 2004). But in this process, irrespective of the nature of the poly anions, the polymer concentration during the nanocomplex forming process must be low, around 0.1% by weight. This can be explained considering that the complex formation, being kinetically controlled, dilution is the only way to limit the interchain cross-linking so as to remain within the colloidal domain. This may represent a limitation for the production of larger amounts of colloidal complex and may hinder the future development of this new family of colloidal materials. Here we report for the first time a new formulation for the synthesis of colloidal polyelectrolyte complexes of chitosan, which addresses this shortcoming and which relies on the control over the polyelectrolyte chains interactions.

2. Experimental

2.1. Materials

Chitosan obtained from chitin squid pens with a relatively high weight-average molar mass and low degree of acetylation (batch 113, *DA* ~1.5%, Mw ~532 000 g mol^{-1}) was purchased from Mahtani chitosan Pvt. Ltd. Prior to use, the polymer was purified by dissolving it at 0.5% (w/v) in a stoichiometric amount of aqueous acetic acid and by filtering the resulting solution successively on membranes (Millipore) of porosity: 3, 1.2, 0.8 and 0.45 μm. Then, the polymer was precipitated with aqueous ammonia until pH 9–10. After repeated washings with de-ionized water, the neutral precipitate was freeze-dried.

The resulting chitosan purified, with a low content of GlcNAc units was *N*-acetylated to obtain a homogeneous series of samples of different *DAs*. The reaction was performed under soft conditions in a fresh solution of acetic anhydride in a water/propanediol mixture (50%/50% w/w) thus allowing the preservation of a statistical distribution of residues within the chains and the only acylation of amine functions according to Vachoud, Zydowicz, and Domard (1997). The products were isolated by precipitation on adding aqueous ammonia followed by repeated washings with de-ionized water, then freeze-dried.

Finally, hydrolysis (Schatz, Domard, et al., 2004) by controlled nitrous deamination was performed to produce low molar weight polymers. Chitosans were dissolved at 0.5% (w/v) in a 0.2 M acetic acid/0.15 M sodium acetate buffer. A 1 g/l sodium nitrite was added to chitosan solutions to obtain a nitrite/glucosamine units molar ratio of 0.1. The reaction was performed under high mechanical stirring for various times. Low molar mass chitosans were recovered by precipitation with ammonia till pH 9–10, followed by repeated washings with deionized water until neutrality and finally lyophilized.

Dextran sulfate was purchased from Sigma Aldrich and was used without further purification. The water content was determined by thermogravimetric analysis (TA Instrument TGA Q500). The molecular characteristics were determined by gel permeation chromatography, according to Schatz, Domard, et al. (2004). The degree of sulfation (the number of sulfate functions per glucosidic unit) was 2.2, as determined by colloidal titration using toluidine blue (Ueno & Kina, 1985).

Table 1
Physicochemical parameters of chitosans determined by ^{1}H NMR and SEC in an acetic acid/ammonium acetate buffer (pH 4.5).

DA (%)	Mw, $\times 10^5$ (g mol^{-1})	Ip
1.5	4.80 ± 0.05	1.8 ± 0.02
9	4.46 ± 0.05	1.7 ± 0.02
	2.73 ± 0.02	1.5 ± 0.01
	2.25 ± 0.02	1.5 ± 0.02
	0.76 ± 0.001	1.3 ± 0.02
	0.24 ± 0.001	1.2 ± 0.02
19	4.32 ± 0.05	1.5 ± 0.02
	3.08 ± 0.03	1.5 ± 0.02
	2.87 ± 0.03	1.5 ± 0.02
	1.07 ± 0.01	1.4 ± 0.02
	0.33 ± 0.02	1.2 ± 0.02
26	3.59 ± 0.05	1.7 ± 0.04
	3.24 ± 0.03	1.7 ± 0.02
	2.78 ± 0.04	1.6 ± 0.05
	1.02 ± 0.01	1.4 ± 0.04
	0.37 ± 0.003	1.3 ± 0.02
27	4.96 ± 0.06	1.6 ± 0.05
	3.14 ± 0.08	1.6 ± 0.07
	1.04 ± 0.07	1.3 ± 0.02
	0.32 ± 0.01	1.2 ± 0.06
32	4.33 ± 0.05	1.5 ± 0.05
33	4.83 ± 0.06	1.5 ± 0.04
39	4.58 ± 0.05	1.7 ± 0.05
	3.51 ± 0.03	1.7 ± 0.02
	3.00 ± 0.02	1.6 ± 0.02
	1.33 ± 0.03	1.4 ± 0.05
	0.52 ± 0.06	1.2 ± 0.1
47	3.96 ± 0.04	1.7 ± 0.03
	1.88 ± 0.04	1.7 ± 0.05
	1.49 ± 0.05	1.5 ± 0.08
	0.52 ± 0.02	1.2 ± 0.06
50	4.62 ± 0.09	1.6 ± 0.03
	3.04 ± 0.04	1.6 ± 0.03
	2.73 ± 0.04	1.6 ± 0.04
	1.78 ± 0.03	1.5 ± 0.04
	0.74 ± 0.001	1.2 ± 0.02

2.2. Methods

2.2.1. Characterization of chitosan

The degree of acetylation was determined by ^{1}H NMR spectroscopy (Brucker Avance III 400 MHz) at 25 °C. Samples were prepared by dissolving 10 mg of chitosan in 1 mL of D2O and 5 μl of HCl. The *DA* value was calculated according to the Hirai et al. method.

The water content was determined by thermogravimetric analysis (TA Instrument TGA Q500). The weight average molar mass Mw and the polydispersity indexes (Ip) were measured by size exclusion chromatography (2500 and 6000 PW TSK gel columns from Tosohaas) coupled online with a differential refractometer (Wyatt Optilab T-rEx) and a multiangle laser light scattering detector (Wyatt Dawn EOS) operating at λ = 633 nm. A degassed 0.2 M acetic acid/0.15 M ammonium acetate buffer with a pH 4.5 was used as the eluent. The flow rate was maintained at 0.5 ml/min. The refractive index increments (dn/dc) were added independently for each degree of acetylation according to a previous study (Schatz et al.). The physicochemical parameters of the chitosan samples are reported in Table 1.

2.2.2. Preparation of polyelectrolytes solutions in presence of different sodium chloride concentrations

Taking into account the initial water content, chitosan was dispersed at various concentrations with a stoichiometric amount of aqueous acetic acid with respect to the free amines group for each chosen degree of acetylation. Dissolution was achieved after overnight stirring. Then, sodium chloride was added to each solution to obtain the expected concentration. Dextran sulfate