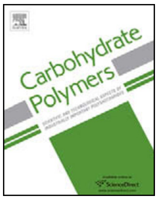
Carbohydrate Polymers 109 (2014) 44–48

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



Self-scrolling ability of differentially acetylated chitosan film

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ARTICLE INFO

Article history:
Received 24 November 2013
Received in revised form 17 March 2014
Accepted 19 March 2014
Available online 28 March 2014

Keywords:
Chitosan
Acetylation
self-scrolling
FT-IR
ATR-IR

ABSTRACT

Chitosan film cast on a glass slide was exposed to acetic anhydride vapor, resulting in an acetylation gradient in the film, with preferential acetylation of the exposed surface. The difference in degree of acetylation between the two surfaces of the peeled film was confirmed by attenuated total reflection infrared spectroscopy. Upon immersion of the film in water, differential swelling occurred because the more highly acetylated surface absorbed less water, and the resulting bending moment caused self-scrolling with the more highly acetylated surface inside. Simultaneous peeling and scrolling of films with a thickness of micrometer order, using dilute aqueous hydrofluoric acid, afforded tightly rolled chitosan microtubes. This simple self-scrolling mechanism is potentially applicable for micro-scale design with various naturally occurring polymers.

1. Introduction

Self-scrolling mechanisms are now widely employed, e.g., in microfluidics, (Prinz & Prinz, 2003; Smith, Xi, Makarov, Mönch, Harazim, & Quiñones, 2012), X-ray optics (Deneke & Schmidt, 2004), drug delivery and encapsulation of living cells (Zakharchenko, Puretskiy, Stoychev, Stamm, & Ionov, 2010). We recently showed that preferential solvent-induced swelling of one side of a polymer film exhibiting non-discontinuous structural heterogeneity in the direction normal to the film surface can also drive self-scrolling (Luchnikov, Sydorenko, & Stamm, 2005). Specifically, we prepared a poly(4-vinylpyridine) (P4VP) film with a gradient of crosslinking degree generated by UV irradiation of one side of the film. In acidic water, the less crosslinked surface of the film swells more than the more cross-linked surface, causing the film to self-scroll and affording tightly rolled scrolls of P4VP containing 10–12 inner layers (Luchnikov, Saito, & Tzanis, 2012). To our knowledge, all "intelligent" polymer films reported so far have utilized synthetic polymers, such as polystyrene, P4VP, etc., derived from fossil hydrocarbons. However, if tightly rolled scrolls of naturally occurring polymers such as cellulose and chitin/chitosan could be produced, we expect that they would have many potential biomedical applications. Therefore, the aim of the present study was to see whether our self-scrolling technique is applicable to a naturally occurring polymer.

Chitosan, (1-4)-2-amino-2-deoxy-<β>-D-glucan, the cationic partially deacetylated derivative of chitin, is a well affirmed biopolymer owing to biocompatibility, biodegradability and absence of toxicity (Muzzarelli, 2012; Muzzarelli et al., 2012). Natural chitosan shows various degrees of acetylation, and the term chitosan is used idiomatically even for partially *N*-acetylated materials. Chitosan has excellent biocompatibility and has been employed in drug delivery systems, (Suzuki, Nakamura, Matsuura, Kifune, & Tsurutani, 1995; Sogias, Williams, & Khutoryanskiy, 2012; Gupta & Jabrail, 2006a,b,2007), as a scaffold of cultured cells for tissue regeneration (Roughley, Hoemann, DesRosiers, Mwale, Antoniou, & Alini, 2006), in tissue engineering (Giri, Thakur, Alexander, Badwaik, & Tripathi, 2012), in targeting strategies for cancer therapy (Patel, Patel, & Patel, 2010), and as an antiseptic agent (Potara, Jakab, Damert, Popescu, Canpean, & Astilean, 2011). Scrollable films would further extend its potential medical applications, and therefore we selected chitosan for the present study.

There are also technical reasons for preferring chitosan: it is soluble in solutions of certain weak acids from which it can be cast as a film quite easily; secondly, its swelling capacity can be modulated via controlled re-acetylation. Chitosan swells and dissolves owing to salt formation, when it assumes the cationic form of a protonated primary amine, but it remains insoluble owing to tight inter- and intra-chain hydrogen-bonds when a majority of acetamido groups are present (Aiba, 1991; Muzzarelli, 1977, 2012; Muzzarelli et al.,

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http://dx.doi.org/10.1016/j.carbpol.2014.03.047
0144-8617/

2012). We considered that if the chitosan film could be differentially acetylated in the direction normal to the surface, the resulting film would exhibit self-scrolling behavior due to differential water uptake at the two surfaces. To obtain heterogeneous acetylation of very thin film, we chose a much milder treatment than that generally used for chitosan (Rowell, Tillman, & Simonson, 1986), based on the method developed for ligno-cellulosic materials (Rowell et al., 1986). For the present purpose, this reaction was carried out in the vapor phase, by exposing chitosan film cast on a glass slide to acetic anhydride ($(CH_3CO)_2O$) vapor, to obtain a film with an acetylation gradient in the depth direction.

2. Materials and methods

Chitosan500 with a degree of acetylation (DA) of ca. 80%, viscosity with 0.5% solution in 0.5% aqueous acetic acid of 200 to 700 CPS, corresponding to the Mv of 220×10^4 to 550×10^4, purchased from Wako Pure Chemical Industries, Ltd., was used. The ash at 600 °C content was 0.2%. Chitosan 500 powder was dissolved in 1 w% aqueous acetic acid by stirring for 1 h at room temperature and a solution of 10 g L^{-1} was prepared. The solution was filtered through a 1G2 glass filter and then dispensed onto a glass slide, which was allowed to dry in an oven at 105 °C overnight.

The chitosan cast film on the glass slide was placed in an airtight container of 200 mL volume together with 5 ml of $(CH_3CO)_2O$. After degassing, the container was heated at 100 °C for 15 min in order to expose the specimen to $(CH_3CO)_2O$ vapor. Then the specimen on the slide was washed in distilled water overnight and dried in oven at 105 °C for another night, to completely remove residual $(CH_3CO)_2O$ in the film.

IR measurements were performed using a Nicolet Magna 860 FT-IR spectrometer in the transmission mode, with a resolution of 4 cm^{-1} and scan number of 32 times. In order to separately measure the $(CH_3CO)_2O$-exposed and unexposed surfaces, attenuated total reflection (ATR)-infrared spectroscopy (IR) was performed with a Micro ATR-IR SPECTRUM2000 (PerkinElmer) under the following conditions: reflection angle 30°, resolution 4 cm^{-1}, scan number 16 times. This enables measurement of a region of the film within a few micrometers from the surface. The exposed surface of the film on the glass slide was measured directly by placing the diamond tip of the instrument on it, while the unexposed surface was measured after carefully peeling the film off the glass slide with tweezers, and then mounting it on a glass slide with the unexposed surface upwards. After peeling off the film, we tested the glass slide for residual chitosan. None was detected, indicating that the film had been successfully peeled off intact. The spectral baselines were corrected by drawing straight lines between 1780 and 1480 and 1230 and 830 cm^{-1}, according to Shigemasa et al. (Shigemasa, Matuura, Sashiwa, & Saimoto, 1996). The DA was compared using the absorbance of the acetyl group bands at 1665 cm^{-1}. For calibration, we used the 1030 and 1070 cm^{-1} bands. We decided to use the values of A_{1665}/A_{1070} and A_{1665}/A_{1030}, which Shigemasa et al. (Shigemasa, Matuura, Sashiwa, & Saimoto, 1996) concluded to show the best linear relations with the DA of chitosan and used them to calculate the absolute value of DA according to the original method. Then the ratio was calibrated as $(A_{1665}/A_{1070}) \times (1070/1665)$ and $(A_{1665}/A_{1030}) \times (1030/1665)$, in order to minimize the influence of the depth of penetration associated with the evanescent field of the IR radiation, with which the depth of penetration associated with the evanescent field of the IR radiation is inversely proportional to the radiation wavelength, i.e., directly proportional to the radiation wavenumber. It should be noted that Shigemasa's formula was derived from the IR spectra of KBr-blended samples, and may not be directly applicable to ATR measurements. Nevertheless, we consider that it is meaningful to compare relative differences between samples measured using the same system.

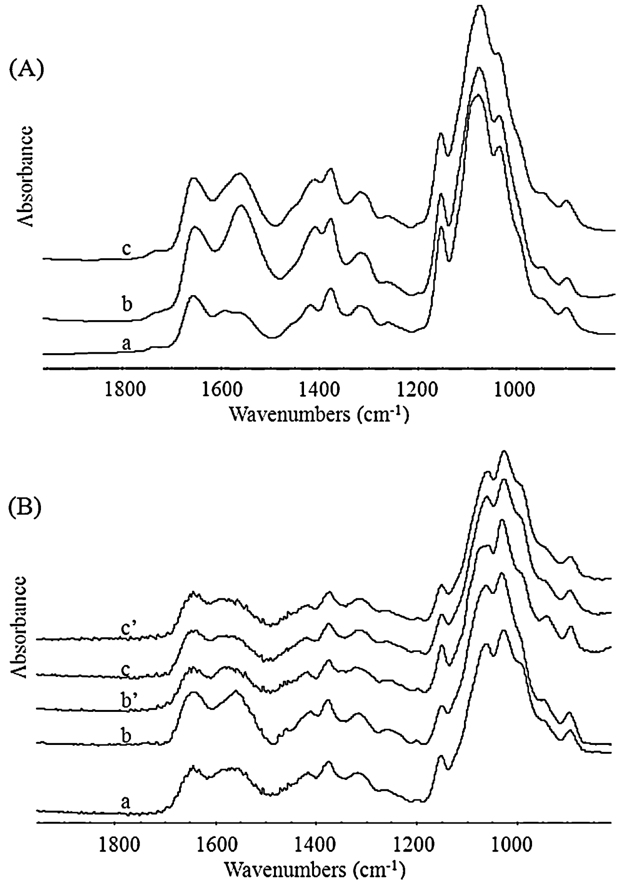

Fig. 1. (A) FT-IR spectra of chitosan films untreated (a), washed in water after $(CH_3CO)_2O$ treatment (b) and washed in ethanol after $(CH_3CO)_2O$ treatment (c). (B) ATR-IR spectrum of a chitosan film of ca. 41 μm thickness untreated (a), washed in water after $(CH_3CO)_2O$ treatment (b, b') and washed in ethanol after $(CH_3CO)_2O$ treatment (c, c'). (b, c) are from the $(CH_3CO)_2O$ -exposed surface and (b', c') are from the unexposed surface.

To examine micro-scrolling from a thin film (about 4 μm thick), which is too fragile to handle with tweezers, simultaneous peeling and scrolling were carried out on the glass slide by placing a drop of 0.25% aqueous HF onto the film, which had a straight edge, previously made by scratching the thin film with a razor. Scrolling of the film was observed in situ with an optical microscope.

The thickness of the films used for ATR-IR measurement was measured with a micrometer (PDN-20 digital gage, Peacock). In the case of films for micro-scrolling, which were no more than several μm thick, the thickness was measured with a scanning electron microscope (SEM).

3. Results and discussion

Examples of FT-IR and ATR-IR spectra of chitosan films are shown in Fig. 1A and B, respectively. Since FT-IR and ATR-IR spectra have different baseline and intensity characteristics, they are discussed separately. The absorbance at 1655 cm^{-1} appeared higher with the $(CH_3CO)_2O$-treated sample (Fig. 1Ab) and $(CH_3CO)_2O$-exposed surface (Fig. 1Bb, c) as compared with the untreated sample (Fig. 1Aa) and unexposed surface (Fig. 1Ba), respectively. Table 1 shows DA indices of the samples before and after exposure to $(CH_3CO)_2O$ vapor. FT-IR measurements showed that the $(CH_3CO)_2O$-treated films have higher absorbance ratios than the unexposed films, indicating that they have higher DA. When

Table 1
DA of chitosan film evaluated in terms of acetyl absorption band ratios.

	Treatment	Washing agent	Thickness (μm)	Measuring surface	DA indices			
					A_{1655}/A_{1070}		A_{1655}/A_{1030}	
ATR-IR	Untreated	–	–	–	0.173	(0.013)	0.156	(0.012)
	$(CH_3CO)_2O$	Water	41	Exposed	0.206	–	0.173	–
				Unexposed	0.180	–	0.148	–
			18	Exposed	0.199	–	0.179	–
				Unexposed	0.180	–	0.161	–
			11	Exposed	0.212	–	0.192	–
				Unexposed	0.193	–	0.173	–
		Ethanol	11	Exposed	0.180	(0.006)	0.155	(0.006)
				Unexposed	0.193	(0.006)	0.167	(0.006)
FT-IR	Untreated	–	–	–	0.138	(0.004)	0.169	(0.006)
	$(CH_3CO)_2O$	Water	–	–	0.221	(0.007)	0.263	(0.020)
		Ethanol	–	–	0.184	(0.027)	0.226	(0.027)

Absorbance ratios were measured at least three times at different locations on the film, except for the water-washed sample. Standard deviations are shown in parentheses.

the two surfaces of films were separately examined by ATR-IR, the $(CH_3CO)_2O$-exposed surface of water-washed chitosan films showed higher absorbance ratios than the unexposed surface, indicating that vapor-phase acetylation had proceeded preferentially at the exposed surface. These results support the idea that the vapor-phase $(CH_3CO)_2O$ treatment of chitosan film on a glass slide produces an acetylation gradient in the direction normal to the film surface. Pyridine, which is normally added as a catalyst and a pH adjuster, was not used here for two reasons. First, removal of pyridine after the reaction could be problematic because of its toxicity, and second, mild reaction in the absence of pyridine was expected to provide a greater degree of control over the structural modification. The difference of DA of the surface could be generated in a mild way without pyridine. In the case of ethanol-washed film, interestingly, an oppositely directed acetylation gradient was found, and the reason for this is discussed below.

This acetylation gradient had two visible consequences. First, the film formed a balloon-like shape (Fig. 2) during washing in water after the $(CH_3CO)_2O$ treatment: a stable skin covers the gel-like swollen part inside. The skin corresponds to the $(CH_3CO)_2O$-exposed surface, which is highly acetylated and less swollen by water. Second, we observed scrolling when a dried film was immersed in water (Fig. 3). A film with the thickness of 11 μm was acetylated with $(CH_3CO)_2O$, then washed in water, and dried at 105 °C overnight. It was inserted vertically into water so that both surfaces were exposed simultaneously. Immediately after immersion the water-washed film scrolled with the exposed surface inside (Fig. 3, right). This is because the more highly acetylated exposed side swelled less, as schematically illustrated in Fig. 4. On the other hand, film similarly treated but washed with ethanol scrolled with the exposed surface outside (Fig. 3, left). In this case, the exposed surface had a lower DA, as shown in Table 1, in contrast to the water-washed film, i.e., the direction of the acetylation gradient was reversed. The reason why the DA of the $(CH_3CO)_2O$-exposed surface was rather lower than that of the unexposed surface may be as follows. The solubility of $(CH_3CO)_2O$ in ethanol is much greater than that in water, so the acetylation reaction would be terminated much more quickly (by removal of $(CH_3CO)_2O$) at the exposed surface than at the deeper parts during washing in ethanol. This implies that acetylation reaction due to residual $(CH_3CO)_2O$ in the film continued even during washing in water. This interpretation is supported by the FT-IR spectra, which indicated that the water-washed film has a higher overall DA than the ethanol-washed film (Table 1). Further investigation is necessary to establish the acetylation mechanism in ethanol, but it is at least clear that the film scrolls with the less acetylated surface

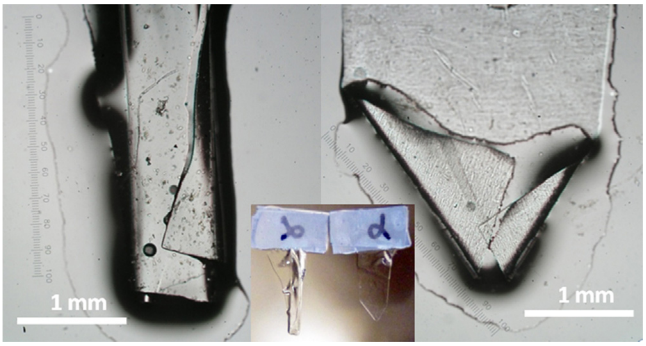

Fig. 3. $(CH_3CO)_2O$-treated chitosan films were immersed in water and mounted on a glass slide with the scrolling side up. Inset photo is the overview with the character "α" indicating the $(CH_3CO)_2O$-exposed surface. The film on the left had been washed in ethanol and that on the right had been washed in water after $(CH_3CO)_2O$ treatment.

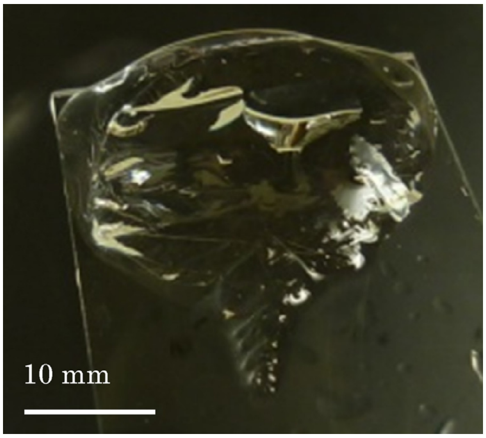

Fig. 2. Chitosan film during washing in water after $(CH_3CO)_2O$ treatment.

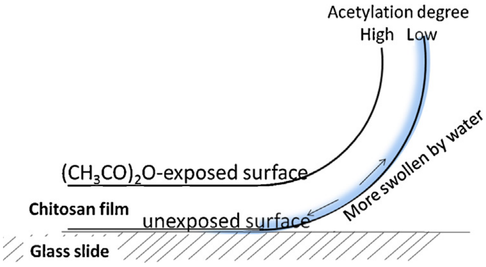

Fig. 4. Schematic illustration of the mechanism of self-scrolling of a differentially acetylated chitosan film.

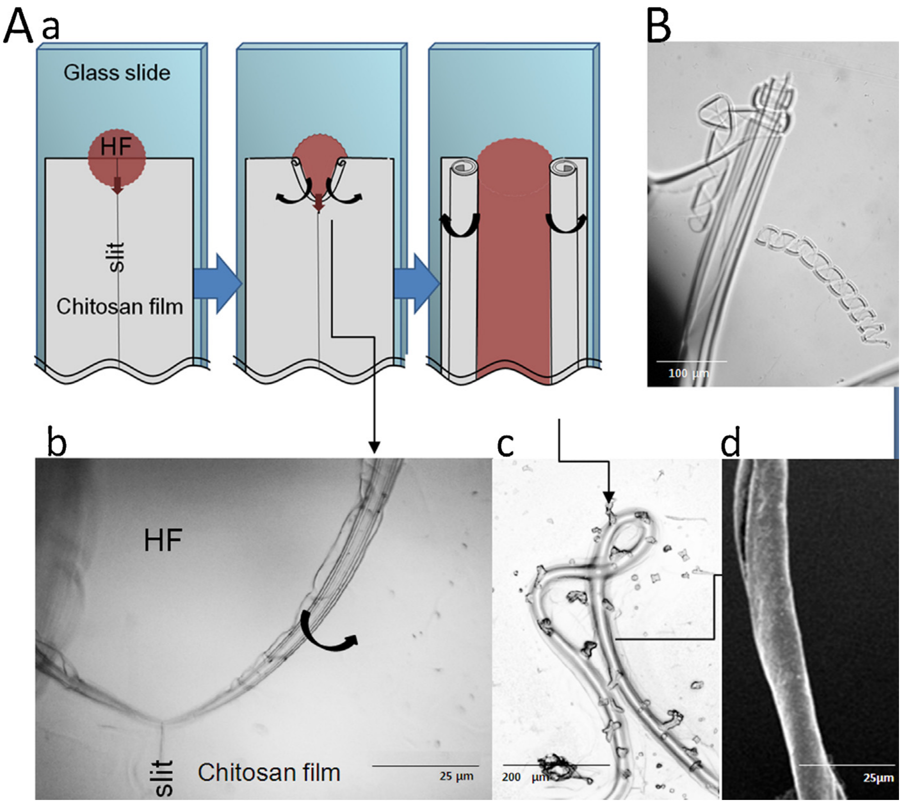

Fig. 5. A: Schematic drawings (a), the optical micrographs at the beginning of micro-scrolling from the slit edges of the chitosan film (b) and after completion of micro-scrolling (c). SEM micrograph of the resultant micro-fiber (d). B: an optical micrograph of spirals from the strap of the chitosan film.

inside in all cases, owing to the principle that a higher level of acetylation makes the material less swellable (Aiba, 1991).

We tried to apply this scrolling to thin films using the method (Luchnikov et al., 2011, 2012) described below. As shown schematically in Fig. 5Aa, when aqueous HF was dropped onto a vertical slit at the cut edge of the film, HF dissolved only the glass. The chitosan film was peeled off from the glass slide and simultaneously swollen. The less $(CH_3CO)_2O$-exposed surface is more swollen, and thus self-scrolling starts from the HF spot, and advances gradually along the slit (Fig. 5Aa middle). Finally, the film is completely rolled up and tubes containing several interior layers are formed (Fig. 5Ab→c). In this case, the film had a thickness of 4 µm and the resultant scroll had a diameter of 10 µm and a length of a few centimeters. This “simultaneous peeling-scrolling method” enabled “micro-scrolling” of films too fragile to manipulate with tweezers. Depending on the thickness and shape of the film, various microstructures were available (Fig. 5B, for example). Other acids, such as HCl and citric acid, were examined, but although they could swell the film, they could not peel the positively charged chitosan film completely off the negatively charged glass base. A less toxic combination of support and peeling-and-swelling agent will be required in future. We believe it will be possible to design tubes of desired diameter, wall-thickness and length, by appropriately controlling the DA-gradient, thickness and initial size of the parent films.

4. Conclusion

By exposing chitosan film cast on a glass slide to acetic anhydride vapor, we obtained chitosan film having an acetylation gradient normal to its surface as an “intelligent material” with self-scrolling ability on exposure to water. ATR-IR measurements confirmed differential acetylation of the two surfaces. When the dried and peeled film was immersed in water, the film self-scrolled with the more highly acetylated surface on the inside, due to its lower water uptake and lower swelling. Simultaneous peeling and scrolling of micrometer-thickness films with aqueous hydrofluoric acid afforded a fibrous material, consisting of tightly rolled scrolls of chitosan.

Acknowledgements

This work was partially supported by Invitation Fellowship Program for Research (FY2012 JSPS, S-12195) of Japan Sociedty for Promotion of Science.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.03.047.

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