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Encapsulation of cinnamaldehyde into nanostructured chitosan films

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ABSTRACT: Recently, bioactive chitosan films featuring naturally derived essential oils have attracted much attention due to their intrinsic antimicrobial properties and applicability to a broad range of applications. Previously, the ability to form thick ($t > 100$ μm), chitosan-essential oil films via solution casting has been demonstrated. However, the fabrication of well characterized ultrathin films ($t < 200$ nm) that contain essential oils remain unreported. Here, we systematically investigate increasing the incorporation of an essential oil, cinnamaldehyde (CIN) into ultrathin chitosan films. Films with and without the surfactant Span®80 were spin-coated. Qualitatively, films exhibited well-defined structural color, which quantitatively ranged from 145 to 345 nm thick. Release studies confirmed that a 6× higher release of CIN was enabled by Span®80 versus the chitosan control films, 30 μg versus 5 μg, respectively. These results suggest that nanostructured chitosan-CIN coatings hold potential to delay bacterial colonization on a range of surfaces, from indwelling medical device to food processing surfaces. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41739.

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INTRODUCTION

Chitosan is a non-toxic, antibacterial, chelating biopolymer produced by the deacetylation of chitin, an abundant organic resource.[1–3] The intrinsic properties of chitosan in conjunction with its solubility in acidic, neutral, and alkaline solutions, make chitosan preferred over chitin for a wide range of bioactive film applications. For instance, in food packaging, chitosan films are used to maintain food quality,[4,5] whereas in wound dressings, the chitosan provides antibacterial activity.[6]

Literature provides abundant examples of thick ($t > 100$ μm), solution-cast chitosan films whose bioactive properties have been augmented via the incorporation of therapeutic, hydrophobic components—antibiotics,[7–10] essential oils,[11,12] and polyphenols.[13] In terms of thinner films, nanostructured chitosan films have predominantly been fabricated via layer-by-layer (LbL) assembly.[14] At low pHs, negatively charged agents including anionic biopolymers,[15] oil-in-water emulsions,[16] and anionic surfactants[17] have been incorporated into positively charged chitosan LbL films. While the LbL process can be automated to enable a rapid large-scale production of multilayer assemblies,[18,19] spin-coating is recognized as the most promising industrial process because it enables the rapid mass production of uniform films with tunable thicknesses, low-temperature fabrication, and high reproducibility.[20–22] Despite these advantages, the studies that have focused on spin-coating chitosan films have been very limited in scope to investigating the environmental conditions while processing,[23] the effect of the biopolymer solution [i.e., blending with poly(ethylene oxide),[24] chemical modification[25]], and their applicability as a sensor for metal ions[26] or aromatic organic compounds.[27]

Currently, there is a poor understanding of the major factors that influence the incorporation of bioactive components, such as essential oils, into spin-coated polymer films. To date, only one manuscript[28] has spin-coated polymer films ($t > 500$ nm) that contained essential oils. Zodrow *et al.*[28] demonstrated that poly(lactic-*co*-glycolic acid) films featuring 0.1 and 1.0% essential oils impaired microbial biofilm formation. Their work confirmed that cinnamaldehyde (CIN) has a strong antimicrobial effect against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*, however, they did not optimize film thickness or quantify the release profile of the essential oil. In the present study, we fabricate and characterize bioactive chitosan ultrathin films featuring CIN, a well-studied essential oil derived from cinnamon bark that has intrinsic antimicrobial, anticancer, and insecticidal activity (Figure 1).[29,30] Increased encapsulation of CIN was enabled using a model surfactant, sorbitan monooleate (Span®80). To the best of our knowledge, this is the first time that an essential oil or a surfactant has been spin-coated into ultrathin ($t < 200$ nm) films. The ability to deliver a tailored quantity of CIN from an ultrathin coating holds potential to protect a range of surfaces from microbial contamination.

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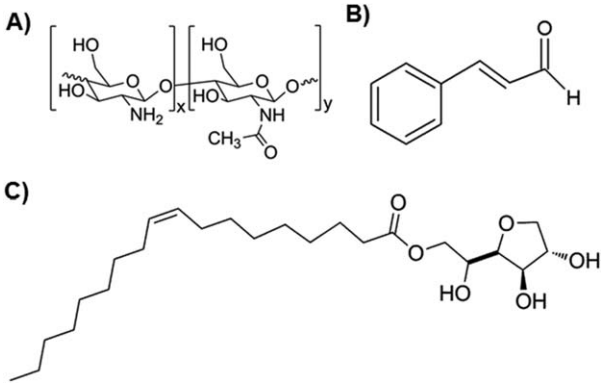

Figure 1. In this work the (A) polysaccharide chitosan and (B) cinnamaldehyde (CIN), an aromatic aldehyde, were spin-coated into ultrathin films in the presence or absence of the surfactant (C) sorbitan monooleate (Span®80).

MATERIALS AND METHODS

Materials and Chemicals

All compounds were used as received. Low molecular weight chitosan ($M_w = 460{,}000$ Da), cinnamaldehyde (CIN, ≥ 93%, FG, $M_w = 132.16$ Da), sorbitan monooleate (Span®80, HLB = 4.3), analytical reagent grade acetic acid (AA), glutaraldehyde (GA, 50 w/v % aqueous solution), deuterium oxide (D_2O), and acetic acid-d_4 (AA-d_4) were obtained from Sigma-Aldrich (St. Louis, MO). Deionized (DI) water was obtained from a Barnstead Nanopure Infinity water purification system (Thermo Fisher Scientific, Waltham, WA).

Chitosan/CIN and Chitosan/Span®80/CIN Solution Preparation and Characterization

A 2.5 w/v % solution of chitosan in 0.5*M* AA was mixed until fully dissolved (24 h at 20 rpm) using an Arma-Rotator A-1 (Bethesda, MA). Emulsions were prepared at organic/aqueous volume ratios of 0, 0.5, 1.0, and 5.0 v/v %, corresponding to CIN/chitosan weight ratios of 0, 0.21, 0.42, and 2.1, respectively. Chitosan/CIN solutions were prepared by adding the various amounts of CIN to the chitosan solution and mixed for an additional 24 h. For the films containing the surfactant, 0.1 w/v % Span®80 was added to the chitosan/AA solution and mixed for 24 h before CIN was added dropwise to the solution at a rate of three drops every 3 min while being continuously mixed on a stir plate. When the final organic/aqueous volume ratio was obtained, chitosan/Span®80/CIN solutions were mixed for an additional 15 min to ensure that a homogeneous solution was obtained. Throughout the mixing process, all solutions had a pH value of 4 and once CIN was added to chitosan, the solutions changed from optically clear to opaque.

Proton nuclear magnetic resonance (NMR, Bruker Avance 400) along with SpinWorks3, an NMR analysis software, were employed to quantitatively determine the degree of acetylation (DA) and degree of substitution (DS) of the chitosan. Solutions for ^{1}H NMR consisted of chitosan/CIN and chitosan/Span®80/CIN emulsions containing 0, 0.5, 1.0, and 5.0 v/v (organic/aqueous)% dissolved in 0.5 M AA-d_4 (600 μL). DA values were determined by taking the relative integrals of 1.7–2.4 ppm over 2.7–4.4 ppm.[31–33] DS values were calculated from the ratio of the integrated resonances of reacted CIN (9.25–9.6 ppm) over glucosamine residues on chitosan (2.7–4.4 ppm).[34,35]

Chitosan/CIN and Chitosan/Span®80/CIN Ultrathin Film Fabrication

Substrates for spin-coating were either silicon wafers (single-sided polished, (100) plane from University Wafer, South Boston, MA) for profilometry or glass coverslips (22 mm × 22 mm × 1.5 mm, Fisherbrand™) for all other characterization. Before spin-coating, silicon wafers were rinsed with water, acetone, and ethanol before 30 min of UV/ozone treatment (UV/Ozone ProCleanerTM, BioForce Nanosciences, Ames, IA) to oxidize the organic material. Cleaned silicon wafers were cut (25 mm × 25 mm) then rinsed again with water, acetone, and ethanol before being dried under an air stream. Glass coverslip were UV/ozone treated for 30 min to oxidize the organic material then rinsed with water, acetone, and ethanol before being dried under an air stream.

Chitosan/CIN and chitosan/Span®80/CIN solutions were dispensed (0.5 mL) onto a substrate and spin-coated (Spin-Coater model SC-100, Smart Coater, St. Louis, MO) at 4000 rpm for 60 s with an additional 1 s ramp time. After spin-coating, remnant AA was allowed to evaporate at room temperature ($T = 21°C$) for 24 h. To ensure their stability for further characterization, films ($n = 6$) were then crosslinked in a vapor chamber (12.2 cm × 9.8 cm × 7.8 cm, Biohit, Neptune, NJ) containing 1.0 mL of glutaraldehyde (GA) liquid at room temperature for 4 h.

Chitosan/CIN and Chitosan/Span®80/CIN Ultrathin Film Characterization

Film thickness was determined using a stylus profilometer (model Dektak 3, Veeco/Sloan, Santa Barbara, CA). Films were scratched using a razor blade before scans of 500 point resolution were run perpendicular to the scratches for lengths of 1000 μm at a rate of 80 μm s^{-1}.[36] The thickness was determined to be the difference between the surface height and the lowest point of the scratch. Tests were performed on three samples of each type of film with three scratches per sample.

Changes in surface hydrophobicity were evaluated using a drop shape analysis system (model DSA 100, KRÜSS, Hamburg, Germany). A DI water drop (5 μL) was advanced at 25 μL min^{-1} on the surface of a film and then subsequently receded at the same rate to obtain the advance and receding contact angles, respectively. Contact angle hysteresis, the difference between the advancing and receding contact angles, was also determined.[37] Each film type was tested in triplicate and three measurements were obtained per film resulting in nine measurements for each film type.

Release of CIN from Chitosan/CIN and Chitosan/Span®80/CIN Ultrathin Films

Films spin-coated onto glass coverslips were cut in half and placed with coated surfaces facing outward in a 10 mL vial filled with 10 mL of DI water. The films were fully submerged. The vials were sealed tightly to prevent evaporation, protected from light, and shaken at 200 rpm at room temperature. After 24 h or 7 days, 8 × 1.0-mL aliquots of release medium (DI water)

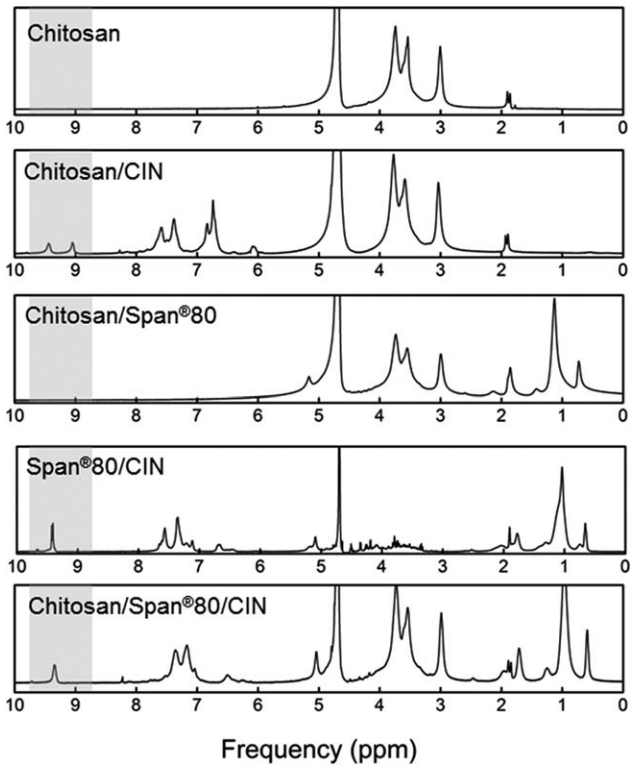

Figure 2. The NMR spectra of (top-to-bottom) chitosan, chitosan/CIN, chitosan/Span®80, Span®80/CIN, and chitosan/Span®80/CIN. Characteristic CIN peaks appear within the grey highlighted region. When mixed with chitosan, unreacted and reacted CIN peaks appear at 9.0 and 9.5 ppm, respectively. However, in the presence of Span®80, the unreacted CIN peak shifts to 9.34 ppm. The concentrations utilized were 2.5% chitosan, 0.1% Span®80 and 0.5% CIN.

from each film sample was tested via UV–visible spectroscopy (Model 8453, Agilent Diode Array, Santa Clara, CA) at an absorbance of 293 nm.[38,39] The absorbance of each aliquot was averaged and related to a CIN concentration based on a standard calibration curve. The calibration curve indicated that the lowest detection limit for CIN was 4 ppm. Total CIN (μg) released per film was calculated based on the 10 mL of released volume. After the 7 day trial, vials were refilled with DI water and retested after another 7 days to determine if there was any further CIN release. Throughout all testing, chitosan and chitosan/Span®80 films were used as controls. Films were tested in triplicate.

RESULTS AND DISCUSSION

Chitosan/CIN and Chitosan/Span®80/CIN Solution Characteristics

Figure 2 displays the NMR spectra of the spin-coating solutions (chitosan, chitosan/CIN, and chitosan/Span®80/CIN), as well as control solutions (chitosan/Span®80 and Span®80/CIN). From the chitosan spectra, it was determined that the chitosan had a degree of *N*-acetylation of 5–7%.[31–33] In the chitosan/CIN spectra, free (unreacted) CIN is present as evident from the characteristic aldehyde peak at 9.0 ppm.[35] The presence of a peak at 9.5 ppm[35] corresponds to the amine group of chitosan reacting with CIN to form a Schiff base. From the ratio of amines reacted with CIN over the total amount of amines,[40] the degree of substitution (DS) was determined to be 13% for chitosan/CIN solutions prepared with either 0.5% or 1.0% CIN (no Span®80). Thus, while a higher initial CIN concentration does not increase the DS, it does correlate to more unattached CIN in the spin-coating solution, as supported by the larger peak of unreacted CIN (at 9.0 ppm). When 0.1% Span®80 was added to the chitosan/CIN (0.5, 1.0, and 5.0%) solutions, the unreacted CIN peak shifted to 9.34 ppm, which has been previously reported.[41] Solutions with Span®80 do not show evidence that a Schiff base has occurred. Span®80 NMR spectra (not shown) displays peaks between 0.5–2.5 ppm and 3.4–5.5 ppm, which would not overlap with the CIN or chitosan-CIN peaks.

Chitosan/CIN and Chitosan/Span®80/CIN Ultrathin Film Characteristics

Spin-coating successfully produced control chitosan films and chitosan films with encapsulated CIN (0.5 and 1.0%) and 0.1% Span®80 with CIN (0.5, 1.0, and 5.0%), Figure 3. Consistent with the work of Zodrow *et al.*,[28] 1.0% CIN was the highest loading of essential oil that could be added to the polymer matrix and spin-coated uniformly without the use of a surfactant. From preliminary experiments that tested Span®80 concentrations ranging from 0.05 to 0.5%, it was determined that 0.1% Span®80 was the lowest concentration that produced visually uniform films; thus, this concentration was held constant. By introducing 0.1% Span®80, the concentration of CIN that could be processed into chitosan films increased to 5.0%. Visually, all films exhibited different structural color (Figure 3), which qualitatively confirmed that the incorporation of CIN and Span®80/CIN altered film thickness.[42]

Statistically, the average thickness of the spin-coated films were all different from one another (Table I). Control chitosan films (no CIN or Span®80) were found to have an average thickness of 195.3 ± 2.5 nm (yellow), comparable to the findings of Murray and Dutcher.[23] Upon the addition of 0.5% and 1.0% CIN, the average thickness of chitosan/CIN films decreased to 159.5 ± 1.0 nm (green) and 144.3 ± 3.7 nm (aqua), respectively. This decrease is likely due to phase separation caused by the addition of CIN. Such separation weakens intermolecular forces, thus decreasing the resistance to centrifugal forces during spin-coating, resulting in thinner films.[43] The initial addition of Span®80 (no CIN) to chitosan resulted in the fabrication of films with an average thickness of 222.5 ± 1.0 nm (magenta). For chitosan/Span®80 films, the addition of (0.5%) CIN initially decreased film thickness while further additions of 1.0 and 5.0% CIN continually increased the overall film thickness to 188.1 ± 2.3 nm (yellow), 258.3 ± 2.8 nm (blue), and 246.5 ± 11 nm (purple), respectively. Thicker films upon CIN incorporation might be due to the addition of surfactant, which helps to stabilize the two phases, lessens separation forces, and strengthens intermolecular forces, thus increasing the resistance to centrifugal forces during spin-coating.

Water contact angle measurements determined that all films were hydrophilic, Table I. This suggests that the exposed surface is primarily chitosan due to the polymer's positively charged free amine groups, rather than the hydrophobic CIN. In general,

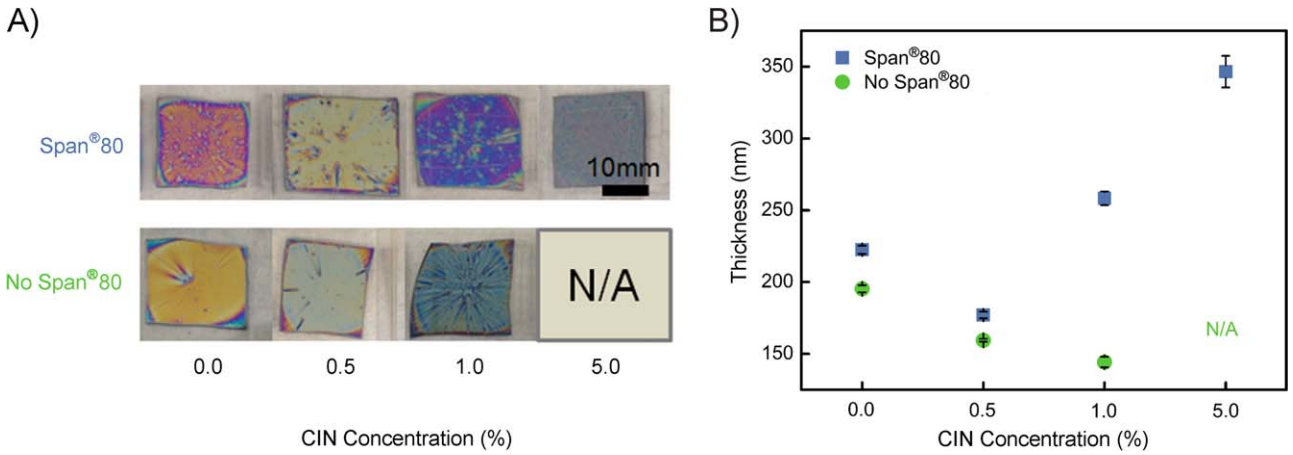

Figure 3. (A) Representative digital images of spin-coated chitosan films containing 0, 0.5, 1.0, and 5.0% CIN without Span®80 (bottom row) and with Span®80 (top row). Visually, the ultrathin films exhibit different structural colors, which qualitatively confirm that they are different thicknesses. (B) Quantitatively, the thicknesses of chitosan films containing CIN and CIN/Span®80 were found to be statistically different from one another. In (A) and (B) concentrations >1.0% CIN could not be spin-coated without the aid of Span®80, as denoted by "N/A." All solutions were spin-coated from 2.5% chitosan solutions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

surfaces that are more hydrophilic are less susceptible to biofouling,[28] which is advantageous for multiple bioactive film applications. The addition of Span®80 to films resulted in a statistically significant increase in the advancing contact angle, as well as a statistically significant decrease in the receding contact angle. Contact angle hysteresis increased for films with Span®80 versus films fabricated without Span®80. Previously, it has been reported that an increased hysteresis correlates with a decreased surface heterogeneity and/or surface roughness,[37,44] both of which have also been reported to increase when a surfactant is present.[45]

Release Characteristics of Chitosan/CIN and Chitosan/Span®80/CIN Ultrathin Films

The quantity of CIN released from chitosan/CIN and chitosan/Span®80/CIN ultrathin films over a 24 h and a 7-day period was determined using UV–visible spectroscopy, Figure 4. After 24 h, chitosan/CIN films fabricated with 0.5 and 1.0% CIN released a statistically equivalent amount of CIN, ~4–5 μg per film or 0.01 μg mm^{-2}. Thus, increasing CIN past 0.5% in chitosan films did not result in additional CIN release. There was no increase in release after 7 days indicating that all "releasable CIN" was released within the first 24 h. However, films fabricated using Span®80 demonstrated an increased release that paralleled the increased CIN incorporation. After 24 h, chitosan/Span®80/CIN films containing 0.5, 1.0, and 5.0% CIN released 8.8 ± 0.3, 17.6 ± 0.6, 29.0 ± 1.0 μg, respectively. In terms of scalability, these release quantities would be the equivalent of 0.02, 0.04, and 0.06 μg mm^{-2}, respectively. Previous reports that fabricated chitosan/CIN macrofilms via solution casting only demonstrated that there was a CIN release through a heightened antibacterial activity. To the best of our knowledge, there are no reports that quantify the release of CIN from chitosan macrofilms.[11,12] Notably, the quantity of CIN released from our ultrathin films is on the same order of magnitude needed for effective antibacterial activity.[28,46] After a 7-day period, there was no appreciable change in CIN release. Thus, Span®80 can effectively be used to incorporate and release higher levels of CIN. To ensure that all "releasable" CIN was released, at the conclusion of the 7-day testing, the samples were drained and refilled with 10 mL of fresh DI water. After 7 additional days, if there was any further CIN release, it was below our detection

Table I. Properties of Chitosan Ultrathin Films: Control, with CIN, and with CIN/Span®80

	CIN (%)	Thickness (nm)	θ_A (°)	θ_R (°)	$\theta_{hysteresis}$ (°)
No Span®80	0	195.3 ± 2.5	56.5 ± 1.1	31.8 ± 1.2	24.7
	0.5	159.5 ± 1.0	60.1 ± 1.0	29.8 ± 0.7	30.3
	1	144.3 ± 3.7	49.7 ± 0.9	29.3 ± 0.9	20.4
Span®80	0	222.5 ± 2.8	73.3 ± 0.4	27.8 ± 0.3	45.5
	0.5	177.1 ± 2.3	66.5 ± 1.4	18.2 ± 0.5	48.3
	1	258.3 ± 4.8	73.0 ± 0.5	19.4 ± 0.7	53.6
	5	346.5 ± 11	69.9 ± 1.2	15.9 ± 0.5	54.0

Provided are the average film thickness, as well as the advancing (θ_A), receding (θ_R), and hysteresis (θ_H) contact angles. Displayed are the average ± standard deviation. All film thicknesses were statistically different from one another. The addition of Span®80 caused statistically significant increases in the advancing contact angle and decrease in the receding contact angle.

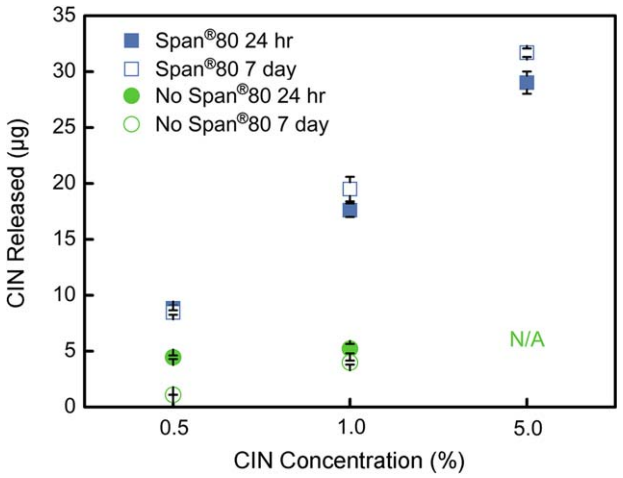

Figure 4. With increasing CIN concentration (0.5, 1.0, and 5.0%) the quantity of CIN released from the chitosan/Span®80/CIN ultrathin films statistically increased. CIN release was the same from the chitosan/CIN (0.5 and 1.0%) ultrathin films. Control chitosan and chitosan/Span®80 ultrathin films did not show any release (not shown). Concentrations above 1.0% CIN could only be spin-coated with the aid of Span®80, as denoted by the "N/A" placeholder. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

limit. The governing mechanism of CIN release from both chitosan and chitosan/Span®80 is most likely swelling-controlled.[47,48] NMR indicated that a majority of the CIN was physically incorporated into the chitosan films and thus, swelling the chitosan would likely allow the CIN to diffuse through the polymer network following Fickian diffusion.[49]

CONCLUSION

We have demonstrated that spin-coating can be used to incorporate and deliver high-loadings of a model essential oil, cinnamaldehyde (CIN), from ultrathin chitosan films. When facilitated by the surfactant, Span®80, up to 5.0% CIN can be encapsulated within chitosan films. Because of the different loadings of CIN, all films fabricated had statistically different thickness, but remained under 350 nm with well-defined structural color. NMR indicated that a majority of the CIN was physically incorporated into the hydrophilic chitosan films. A 6× higher release of CIN was enabled using Span®80. These natural plant and polysaccharide based bioactive films hold potential for use as bioactive coatings in food packaging and on indwelling medical devices.

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