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Layer-by-Layer Assembled Thin Films and Microcapsules of Nanocrystalline Cellulose for Hydrophobic Drug Delivery

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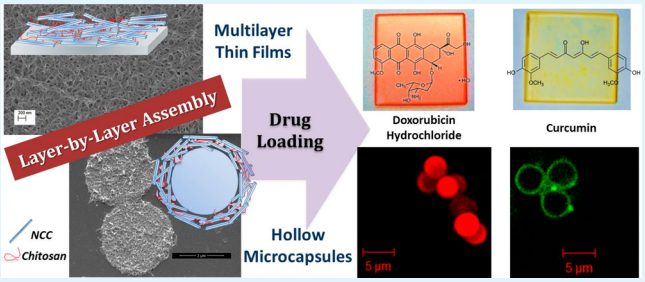
Supporting Information

ABSTRACT: A layer-by-layer (LbL) approach has been employed for the fabrication of multilayer thin films and microcapsules having nanofibrous morphology using nanocrystalline cellulose (NCC) as one of the components of the assembly. The applicability of these nanoassemblies as drug delivery carriers has been explored by the loading of an anticancer drug, doxorubicin hydrochloride, and a water-insoluble drug, curcumin. Doxorubicin hydrochloride, having a good water solubility, is postloaded in the assembly. In the case of curcumin, which is very hydrophobic and has limited solubility in water, a stable dispersion is prepared via noncovalent interaction with NCC prior to incorporation in the LbL assembly. The interaction of various other lipophilic drugs with NCC was analyzed theoretically by molecular docking in consideration of NCC as a general carrier for hydrophobic drugs.



KEYWORDS: *layer-by-layer, hydrophobic drug delivery, NCC, multilayers*

INTRODUCTION

Since the discovery of nanocrystalline cellulose (NCC) in 1949 by Bengt G. Rånby,[1] it has found potential applications as reinforcing nanofiller material[2,3] due to its high elastic modulus and tensile strength,[4,5] with the added advantage that it can be used for biobased nanocomposites. Owing to its excellent mechanical properties, NCC had been extensively explored in various fields, such as polymer electrolytes, packaging, antireflective materials, and solid iridescent films.[6,7] However, recent interest in the application of NCC as a biomaterial in the biomedical field, mainly in tissue engineering[8,9] and drug delivery systems,[10,11] has grown as a result of its biodegradability and low cytotoxicity.[8,10] Nanocrystalline cellulose, also referred to as cellulose nanocrystals or cellulose nanowhiskers, are crystalline rod-shaped particles that are extracted from naturally occurring cellulose fibers by acid hydrolysis.[4] The acid hydrolysis by sulfuric acid results in the functionalization of the surface of NCC with anionic sulfate groups.[12] The presence of these sulfate groups leads to a stable colloidal suspension in water, which makes NCC useful for various biological applications.[8−11]

Among the various self-assembly approaches reported for the fabrication of drug delivery carriers, the layer-by-layer (LbL) technique has gained phenomenal interest among researchers for the fabrication of multilayer thin films for transdermal drug delivery and surface coating of biomedical implants,[13−16] as well as microcapsules for encapsulation and sustained release of therapeutics,[17,18] because of its low cost, simplicity, and versatility.[19] The LbL technique was first realized and established by Decher et al. in the 1990s[20,21] based on the pioneering work of Iler in 1966[22] and generally involves sequential adsorption of oppositely charged polyelectrolytes on a substrate with the assembly stabilized by electrostatic interaction. The approach is versatile in terms of the components of assembly and the type of interaction; not only polyelectrolytes but uncharged polymers, micelles,[23] dendrimers,[24] nanoparticles,[25,26] can be assembled via hydrogen bonding[27] or covalent bonding.[28] In the past, fabrication of multilayer thin films of NCC with a positively charged polyelectrolyte via LbL assembly approach has been attempted with emphasis on the mechanical and optical properties of the film.[7,29−31] On account of the aforementioned biological properties of NCC, we have constructed LbL thin films using chitosan as the complementary polymer for the purpose of drug delivery. Chitosan's properties of biocompatibility and biodegradability have resulted in its widespread use in biological application.[32] In addition, the mucoadhesive property of chitosan makes it suitable for drug delivery and for the preparation of scaffolds for tissue engineering and wound healing.[32] The LbL approach is further extended to a spherical sacrificial template for the preparation of hollow microcapsules. The aqueous cavity obtained on dissolution of the template serves as a reservoir for small drug molecules or macromolecules. The polymeric shell can also be separately loaded

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with drugs. Although the LbL approach is widely used for the deposition of polymers, the versatility of the method does not restrict the use of three-dimensional entities like nanoparticles, even on spherical template. Here, we have successfully used this method for the fabrication of microcapsules of NCC with nanofibrous and porous membranes.

One of the challenges of drug delivery is the delivery of water-insoluble drugs. Many of the hydrophobic drugs showing high potency remain in the pipeline because they suffer from lack of means for administration in the body.[33] Various drug delivery carriers designed to address this issue include micelles of amphiphilic block copolymers,[34] liposomes,[35] nanoparticles,[36] pro-drug approach,[37] and conjugation with proteins,[38] and many of these structures have been incorporated in LbL assembled thin films.[37−39] These structures can also be utilized for the fabrication of microcapsules, which is more precarious because the stability of assembly during the core dissolution becomes a concern and the leaching out of the encapsulated drug has to be avoided. Recently, Olivier et al. reported that NCC can be used for solubilization of extremely hydrophobic carbon nanotubes.[40] Encouraged by their study, we have employed NCC as a carrier for hydrophobic drugs. We have used curcumin, which is known for various medicinal uses,[41,42] as a model hydrophobic drug and have shown that NCC can favorably interact with curcumin. The conjugates of curcumin with NCC are further employed for the fabrication of multilayer thin films and microcapsules. Molecular docking studies show favorable interaction of NCC with various other water-insoluble drugs. Therefore, the reported approach for the preparation of hydrophobic drug carriers can be extended to other lipophilic drugs as well.

EXPERIMENTAL SECTION

Materials. Chitosan (molecular weight, 120 000; degree of deacetylation, 85%) and polystyrenesulfonate (PSS), doxorubicin hydrochloride (Dox·HCl) and curcumin were procured from Sigma-Aldrich. Melamine formaldehyde (MF) particles (size 3.3−3.5 μm) were obtained from GmbH (Berlin, Germany). Acetic acid, hydrochloric acid (HCl), and sodium hydroxide were obtained from Qualigens Fine Chemical. Milli-Q water with a specific resistance of 18 MΩ·cm was used for all the studies. NCC was provided by Alberta Innovates Technology Futures.

Layer-by-Layer Self-Assembly of Nanocrystalline Cellulose (NCC) and Chitosan (CH). Chitosan solution was prepared by dissolving 50 mg of chitosan in 10 mL of 1% acetic acid, which was then diluted to 50 mL with water. The pH of chitosan solution was adjusted to 6 using NaOH for the assembly formation. The substrates (quartz plates and glass coverslips) were cleaned by treating overnight with piranha solution (3 H_2SO_4/1.6 H_2O_2) and then washed several times with Milli-Q water, followed by drying under nitrogen flow. The treatment with piranha activates the substrate for deposition of polymer by making the surface hydrophilic and generating negative potential. The first layer was deposited electrostatically by immersing the substrate in CH solution (1 mg/mL) overnight. The first layer is always CH at pH 3.2; at this acidic pH, more number of amine groups are protonated, rendering chitosan a net positive charge. The substrate was then washed with Milli-Q water to remove the unadsorbed polymer and then dried gently under nitrogen flow. The next layer was deposited by immersing the substrate in NCC dispersion (0.5 mg/mL) for 6 h, followed by washing and drying steps. Subsequent layers of CH and NCC were alternately deposited by repeating the aforementioned steps with a dipping time of 30 min for each layer. The multilayer assemblies are designated as $(CH/NCC)_n$ where n denotes the number of NCC deposition steps.

Loading and Release of Doxorubicin. Doxorubicin (Dox) was loaded in $(CH/NCC)_{20}$ films by incubating the films overnight in a 0.2 mg/mL Dox·HCl aqueous solution. The films were then kept in Milli-Q water for 1 h and rinsed with water several times to remove the unabsorbed drug. The loading was confirmed from the UV−vis absorbance spectra of the loaded films, showing characteristic absorbance of Dox in the region 480−500 nm.

The release studies were performed by immersing the films in 5 mL of PBS solution (pH 7.4 and 6.4) maintained at 37 °C. The films were removed from PBS solution after regular intervals of time, washed with Milli-Q water, and dried under gentle nitrogen flow. The absorbance spectrum of the film was recorded before the film was reimmersed in PBS solution. Each time, the PBS solution was replaced with a fresh solution to maintain the sink condition. The percentage of Dox released is calculated as

$$\%\text{Dox released} = \frac{C_0 - C_t}{C_0} \times 100 = \frac{A_0 - A_t}{A_0} \times 100 \quad (1)$$

C_0 is the initial concentration of Dox in the thin film and C_t is the concentration of Dox in the film after time, t, of incubation in PBS solution. C_0 and C_t can be replaced by their absorbance A_0 and A_t, respectively, in the above equation. The absorbance spectra of release media after each release were also recorded to analyze the amount of Dox released.

Curcumin-Loaded NCC and Its LbL Assembly. First, 50 mg of NCC was dissolved in 5 mL of Milli-Q water (pH 7). Then, 200 μL of curcumin solution in ethanol (5 mg/mL) was added dropwise to the NCC solution under stirring. The dispersion was kept overnight under stirring to remove ethanol and then diluted with Milli-Q water to 100 mL. The dispersion was kept for a day for the unabsorbed curcumin to settle; the unabsorbed curcumin was then separated by decantation. The curcumin-NCC (Cu-NCC) dispersion was characterized by UV−vis spectroscopy, showing an absorbance maximum at 445 nm. The LbL-assembled thin films of Cu-NCC with chitosan were fabricated following the same procedure as mentioned earlier for CH/NCC. The release of curcumin was performed in PBS buffer at pH 7.4, following the same protocol mentioned in the previous section for the release of Dox.

Preparation of Coated Particles and Microcapsules. The positively charged MF particles (zeta potential +53.5 mV) were used as a sacrificial template for the preparation of microcapsules. First, 20 μL of 10% dispersion of MF particles were dispersed in 1 mL of negatively charged NCC and were shaken for 2 h. The particles were then centrifuged at 3000 rpm for 3 min, and the precipitate was washed twice with Milli-Q water. Subsequently, five layers of CH and NCC were deposited alternately in the similar manner with time of incubation reduced to 30 min. The final layer was kept as NCC. Microcapsules were obtained by dissolving the MF core in 0.1 M HCl solution for 15 min. The microcapsules were collected by centrifugation at 7000 rpm for 6 min and washed twice with Milli-Q water. For loading of Dox, microcapsules were incubated overnight in 0.1 mg mL^{-1} Dox·HCl solution under gentle shaking. The loaded microcapsules were washed by centrifugation before being observed under confocal microscope. Microcapsules containing curcumin were prepared by using Cu-NCC instead of NCC for the LbL assembly.

Computational Studies. The model for NCC was prepared from the crystal structure of cellulose Iβ, consisting of 18 glucan chains of 8 glucosyl residues each and arranged in four layers.[43] The model exposes six phases, namely, $(\bar{1}00)$, (110), (010), (100), $(\bar{1}10)$ and $(0\bar{1}0)$; of these, (110) and $(\bar{1}10)$ are the most hydrophilic faces. (100) and $(\bar{1}00)$ are comparatively hydrophobic faces, having a comparatively lower number of hydrogen bond acceptors and donors (Supporting Information, Figure S1).

Molecular docking studies in which the NCC Iβ model was taken as receptor and ligands were prepared using Chem Axon were performed using Hex 8.0. The docked poses were energy minimized with the OPLS minimization method.

Characterization. The growth of the assembly was followed by quartz crystal microbalance (QCM) measurements. AT-cut quartz crystal with gold electrode coated on both sides was used. The gold surface was first modified by mercaptopropionic acid (MPA) to render

a negative charge to the surface by incubating overnight in ethanolic solution of MPA. The surface of the electrode was rinsed several times with ethanol and dried under gentle flow of nitrogen followed by vacuum drying to ensure complete removal of solvent. The first layer of polymer was deposited by incubating in CH solution (pH 3, 1 mg/mL) followed by rinsing with Milli-Q water and drying in similar fashion. The subsequent layers of NCC and CH (pH 6) were deposited alternately in a similar fashion. The QCM resonance frequency was measured after each deposition by an electrochemical quartz crystal microbalance (EQCM) oscillator (CH Instruments, Inc.). The change in the frequency of oscillation with each deposition was plotted.

UV−vis absorbance spectra were recorded on a PerkinElmer Lambda 35 UV/vis Spectrophotometer.

Electrophoretic mobilities were determined from a PALS Zeta Potential Analyzer Ver 3.54, (Brookhaven Instruments Corp.) and were converted to zeta potential (ζ) using the Smoluchowski equation. All experiments were performed at 25 °C. The instrument was operated at 4.00 V with the field frequency of 2.00 Hz. The results were averaged over 3 runs each consisting of 20 cycles.

Morphologies of thin films and microcapsules were observed with Scanning Electron Microscope (SEM). Samples were dried overnight under vacuum to ensure complete removal of moisture and were gold sputtered prior to imaging. The images were recorded on an ULTRA 55 field emission scanning electron microscope (Karl Zeiss).

Optical profilometry was used for determining the thickness of the film. A step was created by gently scratching off the film, and the step height (corresponding to the thickness of the film) was measured using a Talysurf CCI Lite non-contact optical profiler.

Confocal laser scanning microscopy (CLSM) images were recorded on a Zeiss LSM 510 META confocal microscope. The excitation wavelengths used were 488 and 543 nm for curcumin and doxorubicin, respectively. The samples were prepared by drop casting on glass a slide and sealed with a coverslip. The samples were prepared just before imaging to avoid drying of the sample.

RESULTS AND DISCUSSION

Layer-by-Layer Assembly of NCC with Chitosan. Nanocrystalline cellulose used in this study carries a high negative zeta potential of −50.83 mV due to the presence of sulfonate groups that were incorporated during the hydrolysis of cellulose with sulfuric acid. The LbL assembly of NCC is performed using, chitosan, a cationic complementary polymer. The amine groups of chitosan are protonated at acidic pH (the amine groups have a pK_a of about 6.5),[44] and the polymer carries a net positive charge. The amine groups can interact electrostatically with the sulphonate groups of NCC. Additionally, provided the structures of NCC and chitosan wherein both the components are composed of the glucose units, there are many functional groups present that can participate in hydrogen bonding (OH···OH and NH···OH) formation and stabilize the assembly formation. The growth of the assembly has been followed by QCM and SEM.

The QCM result shows a decrease in the frequency of oscillation of the crystal with deposition of either component due to an increase in the mass (Figure 1). The growth has been followed for five bilayers, and consistent reduction in the frequency is observed, indicating successful deposition of both components at each step. The reduction in frequency is greater for NCC deposition due to more mass of NCC compared to CH. The growth mechanism of LbL assembly of nanoparticles differs from that of polymers and involves two modes of growth: (1) lateral growth, that is, an increase in the surface coverage of particles on the surface of substrate, and (2) normal growth mode or the vertical growth determining the thickness of film.[45,46] It cannot be concluded from QCM studies whether

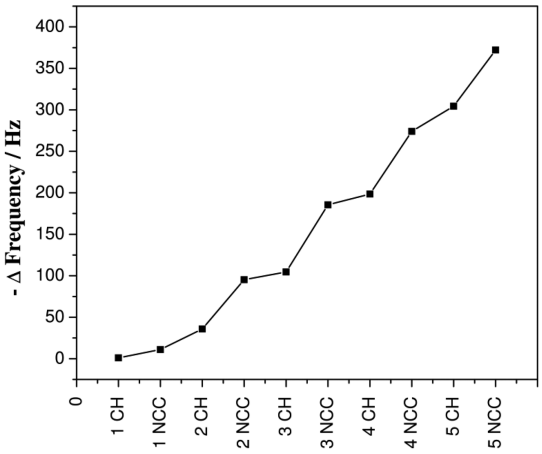

Figure 1. Layer-by-layer assembly of NCC with CH monitored by quartz crystal microbalance (QCM).

the deposition of particles is lateral or vertical; however, the growth mechanism can be perceived from SEM studies. It is observed from SEM images of surface of thin films that surface coverage is achieved in relatively fewer deposition cycles (Figure 2). There is a drastic increase in the surface coverage of

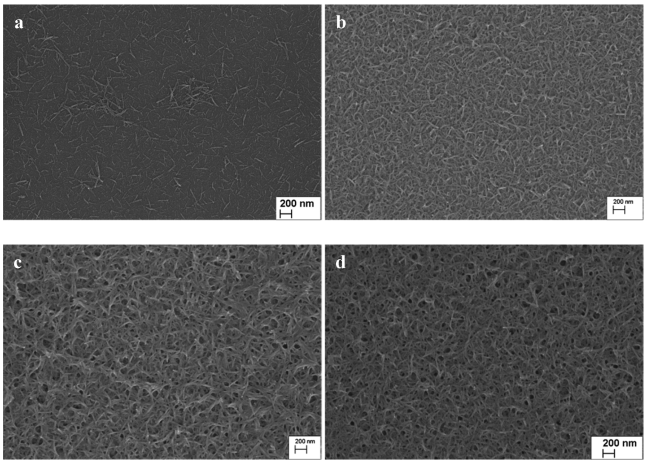

Figure 2. SEM micrographs showing the surface of LbL assembled thin films of chitosan and NCC: (a) $(CH/NCC)_1$, (b) $(CH/NCC)_5$, (c) $(CH/NCC)_{10}$, and (d) $(CH/NCC)_{20}$.

NCC from the first to the fifth layer deposition, and a uniform surface coverage is obtained at fifth layer deposition, unlike the other LbL assemblies reported for nanoparticles with high zeta potential which suffers from interparticle repulsions.[25] The SEM images of the 10th and the 20th layer film evidence the vertical growth of the assembly along with lateral growth. The average thickness of a 20-bilayer film was found to be 606 ± 49 nm from the cross-sectional SEM image (Figure 3). The optical profilometry studies show an average thickness of 600 ± 51 nm measured over six different places.

Loading and Release of Doxorubicin. Doxorubicin is a potential anticancer drug, and in literature, various reports exist on drug formulation of doxorubicin, such as nanoparticles and LbL systems to reduce the associated side-effects.[25,47] In the present study, the hydrochloride salt of doxorubicin (Dox·HCl), which has better solubility in water than the free base, has been used to load the multilayer thin films of NCC by diffusion from its aqueous solution. There are ample free

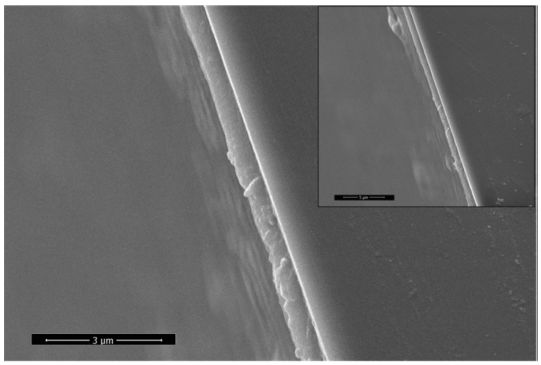

Figure 3. Cross-sectional SEM image of thin film of $(CH/NCC)_{20}$ (scale is 3 μm). (Inset) Lower magnification image (scale is 5 μm).

hydroxyl and sulfonate groups on NCC that can interact with doxorubicin via hydrogen bonding and electrostatic interaction, respectively. The loading of doxorubicin has been confirmed from the UV−vis absorbance spectra of the loaded film where the signature corresponding to doxorubicin appears in the 480−500 nm region (Supporting Information, Figure S2). The films are porous because of their nanofibrous morphology, and hence, it can be anticipated that drug molecules can reach the inner layers, resulting in high loading.

Phosphate-buffered saline (PBS) has been used to mimic the physiological conditions for the drug release studies. The release media used are maintained at a pH of 7.4 (the physiological pH) and at a slightly acidic pH of 6.4, which has importance in anticancer drug delivery due to the acidic extracellular pH of cancerous cells. The release of Dox has been conducted over a period of 9 h and shows a sustained release profile at both the pH (Figure 4a). The sustained release profile of the drug delivery system allows prolonged availability of the drug at the site of action. The percentage release of Dox at pH 6.4 becomes higher than that at pH 7.4 over a longer time period. The higher release of Dox at acidic pH can be ascribed to greater solubility of the drug in water due to protonation of the amine group, which aids the diffusion process.

The drug release mechanism can be elucidated by fitting the release data with Korsemeyer−Peppas semiempirical model, which indicates a linear variation of the percentage release with time on a log−log plot (Figure 4b).[48,49] The release exponent, n, which is obtained from the slope of the linear fitting, characterizes the release mechanism. A value of $n \leq 0.5$ corresponds to Fickian diffusion release of drug from thin films, which is associated with concentration gradient. A value of $0.5 < n < 1.0$ indicates non-Fickian (anomalous) release mechanism, where the drug is released by both diffusion process and erosion of matrix. The value of n was found to be 0.32 and 0.44 for pH 7.4 and 6.4, respectively, which indicates Fickian diffusion of doxorubicin from the matrix in both the cases. The amount of Dox released has been quantified from the absorbance spectra of the release media. The data can be corroborated to the percentage release calculated from the absorbance of film, showing a greater amount of Dox being released at acidic pH of 6.4.

Loading of Curcumin. Although NCC is predominantly hydrophilic due to the presence of a large number of hydroxyl groups, in addition to the sulfonate groups introduced during the preparation, the amphiphilic nature of NCC is evinced by its ability to stabilize oil-in-water Pickering emulsions.[50] This can be attributed to the presence of planes that have predominantly C−H groups and contribute to the hydrophobic character. This has been established by the interaction of cellulose-binding module (CBM) of cellulases, where the planar strip of hydrophobic amino acids of the cellulases (tryptophan, phenylalanine, or tyrosine) anchor selectively on a particular surface of cellulose. The amphiphilic nature of NCC gives us the opportunity to stabilize various hydrophobic drugs in water. In the present report, we have used curcumin as the model drug. Curcumin is a natural polyphenol and is a potent drug known for its therapeutic anti-inflammatory, anticarcinogenic, and antioxidant properties.[41,42]

Unlike Dox·HCl, curcumin has limited solubility in water and cannot be loaded in the LbL assembly by the conventional diffusion method. Hence, the conjugates of curcumin with NCC are prepared by physical mixing of the two prior to LbL assembly formation. The binding of curcumin does not affect the sulfonate groups on NCC, as is evidenced from zeta potential measurement. The zeta potential of NCC is not perturbed on interaction with curcumin; curcumin-NCC (Cu-NCC) shows zeta potential of −50.68 mV, similar to that of native NCC, thereby ensuring good dispersibility of Cu-NCC in water. Cu-NCC is characterized by UV−vis absorbance spectroscopy, showing the absorbance maximum of curcumin at 445 nm (Supporting Information, Figure S3).

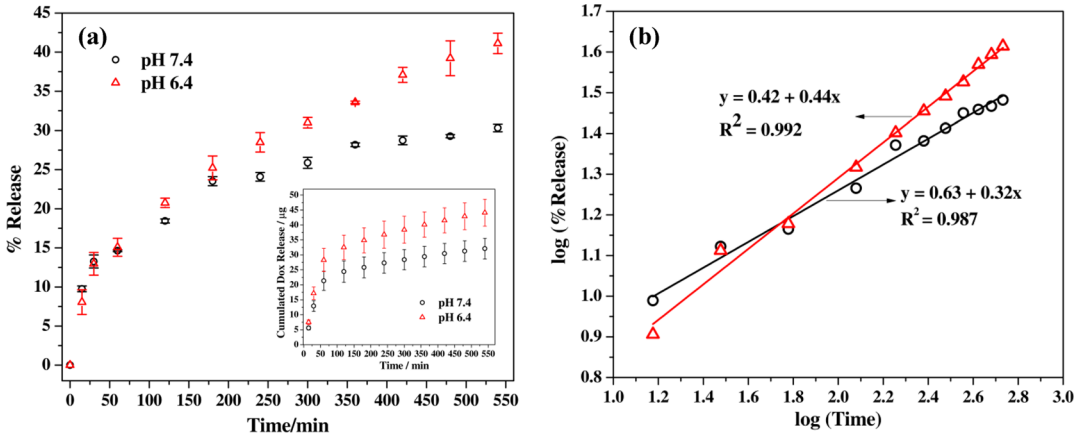

Figure 4. (a) Release profile of doxorubicin from Dox-loaded $(CH/NCC)_{20}$ thin film at 37 °C using PBS buffer (pH 7.4 and 6.4) as release media; (inset) cumulated amount of Dox released in the buffer solution. (b) Korsmeyer model fit of the release profile.

The LbL films of Cu-NCC with chitosan were prepared following a procedure similar to that mentioned in the previous section. The presence of the signature of curcumin in the visible region enabled us to monitor the growth of the assembly, as shown in Figure 5. The increase in the signature of curcumin

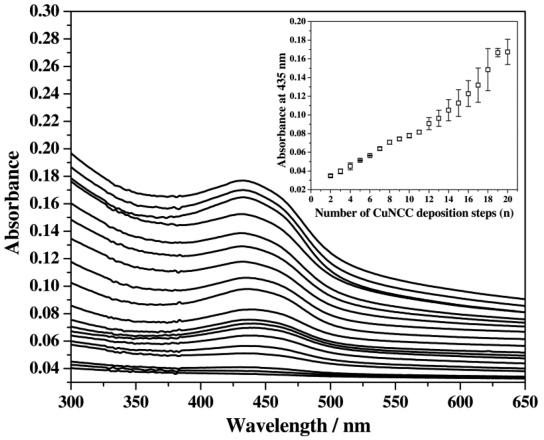

Figure 5. UV−vis spectral analysis for monitoring the growth of layer-by-layer assembly of CH/Cu-NCC thin film. (Inset) The increase in the absorbance with deposition of Cu-NCC.

with increasing deposition steps of Cu-NCC is observed. The amount of curcumin loaded in 20-bilayer films is quantified and is found to be 1.74 ± 0.014 μg cm^{-1}. The release of curcumin has been performed with $(CH/Cu\text{-}NCC)_{20}$ film using PBS buffer of pH 7.4 as the release medium. The sink condition ensures that there is no saturation of curcumin in the release medium, and the concentration difference is maintained for the diffusion of curcumin into the medium. The release profile is a sustained release type similar to that reported in the literature for hydrophobic drugs (Figure 6).[38] The release kinetics was fitted with Korsmeyer model, and a release exponent of 0.22 was obtained.

Interaction of NCC with Various Hydrophobic (Water-Insoluble) Drugs. In the model of NCC, the plane (100) comprising of three glucan chains is the most hydrophobic due to exposure of CH moieties and hence has fewer H-bond forming groups (Supporting Information, Figure S1). The plane $(\bar{1}00)$, lying opposite to (100), has the same hydrophobicity due to same arrangements of glucose units. We have experimentally shown the binding of doxorubicin and curcumin with NCC and theoretically modeled this by molecular docking. Doxorubicin binds at the hydrophobic plane $(\bar{1}00)$ (Figure 7a). The types of interactions involved in this binding

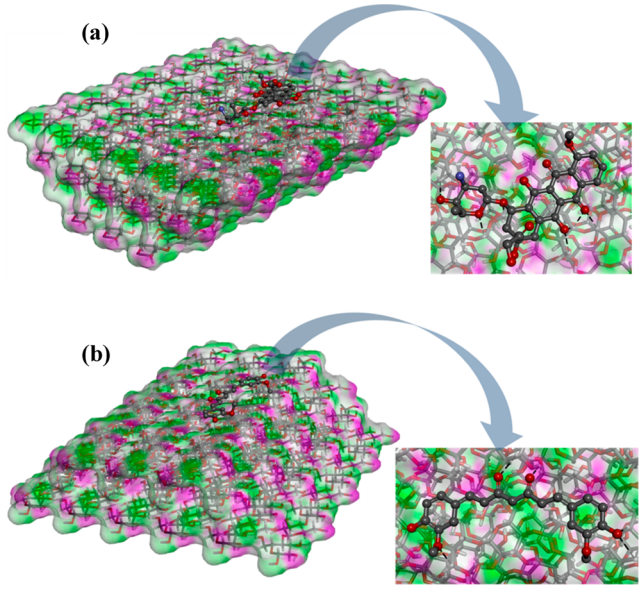

Figure 7. Docking poses of (a) doxorubicin and (b) curcumin on NCC. The chemical skeleton is represented as (gray) carbon and (red) oxygen. In the model of NCC, the surface is represented as (pink) hydrogen bond acceptors, (green) hydrogen bond donors, and (white) hydrophobic patches. Molecular docking shows adsorption of doxorubicin on face $(\bar{1}00)$ and adsorption of curcumin on face (100). The magnified images show the specific interactions of Dox and curcumin with NCC.

are hydrogen bonding (both CH···O and OH···O) along with a significant contribution from van der Waals interactions. Curcumin binds preferentially at (100) plane (Figure 7b). Other lipophilic drugs show binding at either (100) or $(\bar{1}00)$ plane when docked with NCC (Table 1). It is noteworthy that the binding energies of these drugs are comparable to that of curcumin. Therefore, it can be anticipated that NCC can also be used as a carrier for these drugs.

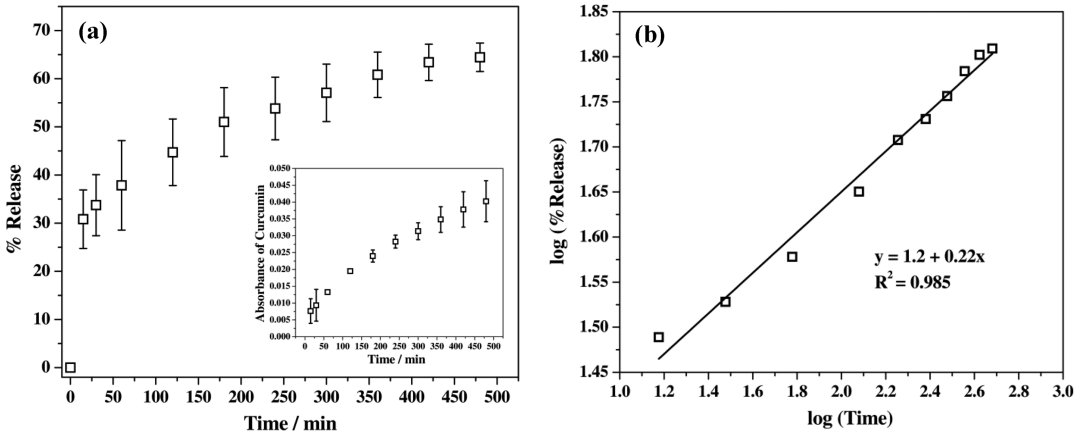

Figure 6. (a) Release profile of curcumin from $(CH/Cu\text{-}NCC)_{20}$ thin film at 37 °C using PBS buffer (pH 7.4) as release media; (inset) the cumulated absorbance of curcumin released in the buffer solution. (b) Korsmeyer model fit of the release profile.

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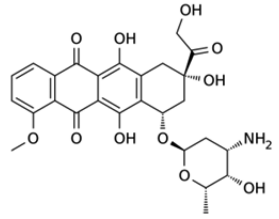
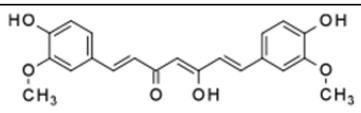
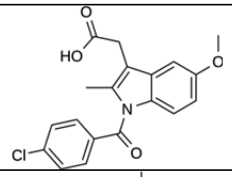
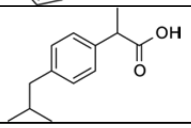
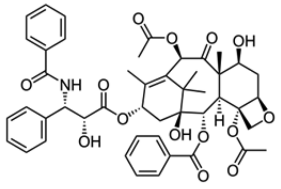
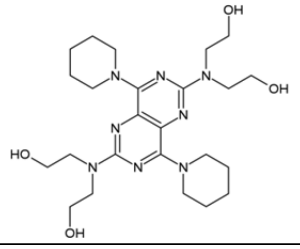
Table 1. Summary of Molecular Docking Results

Drug/Therapeutic Agent	Structure	logP	Binding Energy	Binding Plane
Doxorubicin		1.3	-382.70	(-100)
Curcumin		3.2	-297.18	(100)
Indometacin		4.3	-262.22	(-100)
Ibuprofen		3.5	-160.60	(100)
Paclitaxel		3.2	-257.10	(100)
Dipyridamole		1.5	-277.10	(-100)

LbL Assembled Microcapsules. Microcapsules of NCC were fabricated by using MF particles as the sacrificial template. The narrow size distribution and optimized dissolution condition make MF particles a popular template for the preparation of microcapsules via LbL assembly.[51] The successful deposition of NCC on the template is confirmed from SEM images (Figure 8a). The highly acidic dissolution condition for the MF particles does not disrupt the assembly and stable microcapsules are obtained after five deposition steps of NCC (Figure 8b). The folds and creases observed in SEM images are the characteristics of collapsed hollow microcapsules. This is due to solvent evaporation while drying supplemented with the low mechanical strength of capsule wall.

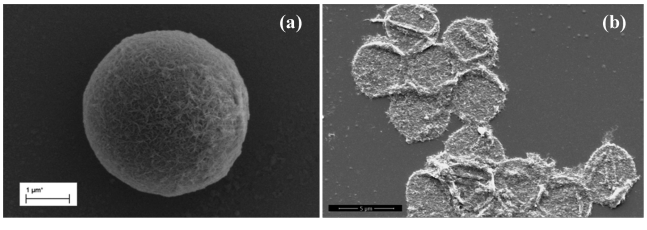


Figure 8. SEM images of (a) MF particle coated with LbL assembled $(CH/NCC)_5$ and (b) hollow microcapsules obtained on dissolution of MF particles.

Unlike previously reported microcapsules of polymers prepared by LbL technique, which have smooth surfaces, the microcapsules of NCC have rugged surface and porous due to nanofibrous nature of NCC. The hollow interior of the microcapsules is used for loading of Dox. Dox has intrinsic fluorescence and hence, the presence of Dox can be visualized under the confocal laser scanning microscopy (CLSM). The CLSM image of MF particles coated with LbL-assembled $(CH/NCC)_5$ shows red fluorescence of doxorubicin on the periphery of the particle due to the loading of Dox in the CH/NCC multilayers (Figure 9a). The Dox fluorescence is observed throughout the hollow microcapsules, indicating loading of Dox in the aqueous interior (Figure 9b).

As discussed in the previous section, the NCC loaded with hydrophobic drug curcumin can be successfully brought in LbL assembly, and thereby, microcapsules for delivering hydrophobic drugs can be prepared by the same method. Microcapsules of Cu-NCC are prepared in the same manner as NCC. The retention of curcumin in the shell of microcapsules during the dissolution by HCl is confirmed

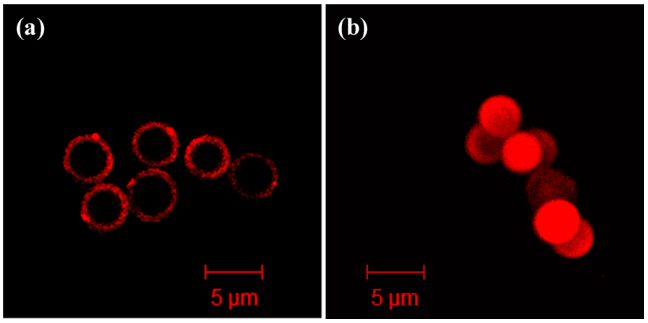


Figure 9. CLSM image of doxorubicin loaded (a) MF particles coated with LbL assembled $(CH/NCC)_5$ and (b) hollow microcapsules. The red fluorescence is due to doxorubicin.

from confocal microscopy (Figure 10), although slight leaching of curcumin in the dissolution medium is observed. In the

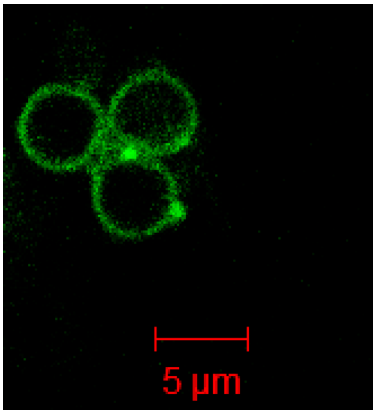


Figure 10. CLSM image of hollow microcapsules prepared via LbL assembly of chitosan and curcumin-NCC (CuNCC) conjugates. The green fluorescence in capsule wall is due to presence of curcumin.

confocal image, the capsule wall is green fluorescent, which confirms the presence of curcumin. Hereby, we have shown two ways of loading microcapsules with drug molecules. Drug molecules having good water solubility like Dox·HCl can be loaded in the aqueous interior by diffusion. However, water-insoluble drugs like curcumin, which cannot be encapsulated by the similar method, can be made water-dispersible by the preparation of drug conjugates with NCC. The drug-bound NCC is used for the fabrication of multilayer hollow microcapsules via LbL technique. This method leads to incorporation of the lipophilic drug in the wall of micro-capsules, leaving the aqueous core of the capsule available for further loading of a hydrophilic drug; thus, the method is useful for dual drug delivery.

CONCLUSION

The complementary electrostatic and hydrogen bonding interaction between positively charged chitosan and negatively charged NCC has been utilized for preparing LbL-assembled thin films for the purpose of drug delivery. The films have a porous nanofibrous morphology and could be loaded with a substantial amount of doxorubicin (an anticancer drug). The loaded drug was released in a sustained manner in physiological condition mimicked by PBS buffer of pH 7.4 and in acidic pH of 6.4. The amount of doxorubicin released at acidic pH is higher, which is commendatory for cancer therapy because of the lower extracellular pH of tumor cells. Though NCC is predominantly hydrophilic in nature, it consists of a surface showing higher hydrophobicity, and hence, it can interact with hydrophobic molecules. In this report, we have demonstrated that NCC can interact favorably with the water-insoluble drug curcumin, and the conjugates of curcumin with NCC form a stable dispersion in water. These conjugates were assembled in LbL fashion for the fabrication of thin films. Curcumin was released in a sustained manner from these films using PBS buffer (pH 7.4) as release medium. The interactions of doxorubicin and curcumin with NCC were investigated by molecular docking studies, and the major interactions were the OH···O and CH···O hydrogen bonding along with contributions from van der Waals interactions. The molecular docking studies on other hydrophobic drugs show favorable interactions, and hence, this approach can be generalized for delivery of other lipophilic drugs as well. The LbL approach was further employed for the fabrication of microcapsules of NCC using MF particle as the sacrificial template. Stable microcapsules were formed after the five-layer deposition of NCC, and the surface of microcapsules have same morphologies as the thin film. The capsules were successfully loaded with Dox, as confirmed from confocal microscopy. The microcapsules containing the hydrophobic drug curcumin in the capsule wall were fabricated via LbL assembly of chitosan with curcumin-NCC conjugates. The leaching of the drug during the dissolution of the template is insignificant, and confocal microscopy shows the presence of curcumin in the capsule walls.

ASSOCIATED CONTENT

Supporting Information

The model for NCC used in computational studies, UV−vis absorbance spectrum Dox loaded thin film and comparison of UV−vis absorbance spectra of free curcumin with NCC-bound curcumin. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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