Preparation, Properties, and Antibacterial Behavior of a Novel Cellulose Derivative Containing Lactam Groups

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ABSTRACT: Lactam groups were introduced onto the backbone of hydroxyethyl cellulose (HEC) to modify properties, such as solubility in organic solvents and solution viscosity and to introduce possible antibacterial activity. Functionalization was achieved using 1-(hydroxymethyl)-2-pyrrolidinone (HMP), and the functionalization reactions were investigated using NMR spectroscopy. The covalent attachment between HEC and HMP was confirmed using ^{1}H-^{13}C correlated NMR experiments. Degrees of functionalization were calculated using integrated ^{13}C NMR spectra, with values of up to 0.9 being demonstrated on the primary alcohol functionality of HEC. The functionalized HECs showed markedly different properties to unfunctionalized HEC, including the ability to swell considerably in water. Functionalized HEC displayed increased thermal stability and reduced solution viscosity compared with unfunctionalized HEC. Moreover, functionalization altered the bacterial adhesion characteristics compared with unfunctionalized HEC. © 2014 The Authors. Journal of Polymer Science Part A: Polymer Chemistry Published by Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2015**, *53*, 68–78

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INTRODUCTION Cellulose, which is a linear assembly of β-D-anhydroglucopyranose units (AGU units), is the most abundant biopolymer on Earth with an estimated 10^{11} tons produced annually.[1–3] AGU units are covalently bonded together via the linkage between the C1 anomeric carbon and the C4 carbon atom providing β-1,4-glycosidic bonds.[4] The main drawback to the use of cellulose for industrial applications is its insolubility in organic and aqueous solvents, with the supramolecular structures within and between polysaccharide strands being primarily responsible for insolubility.[3,5–7] Chemical modification of the hydroxyl groups at C2, C3, and/or C6 positions of cellulose has been developed to confer novel properties, including hydrophilic, antimicrobial, and thermoplastic character, where the degree of substitution (DS) and distribution of the substituents determines the modified cellulose structure and behavior. For cellulose-based materials, DS represents the average number of modified hydroxyl groups per AGU unit, where DS values can range from 0 to 3.[8,9] Hydroxyethyl cellulose (HEC) is a widely used, chemically modified cellulosic material that is produced from the etherification reaction between cellulose and ethylene oxide. HEC is defined by both DS and molecular substitution (MS) which is the number of ethylene oxide groups. HEC shows increased aqueous solubility compared with cellulose because the modification of the C6 and C3 positions of cellulose disrupts, respectively, the inter- and intrachain H-bonding networks. HEC-type materials have a wide range of applications as thickeners, cosmetic film formers, and stabilizers and suspending agents in the paint industry.[10–12] However, HEC's insolubility in organic solvents and the absence of chemically distinct functional groups limit considerably its use in industry.

The beneficial properties of lactam-based polymers are well-known through the use of materials such as poly(*N*-vinylpyrrolidone) (PVP). PVP is a biocompatible polymer which is commonly

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prepared via radical polymerization of *N*-vinylpyrrolidone.[13] The key property of PVP is its solubility in both organic and aqueous solvents. Its solubility is due to the presence of the lactam amide group which provides both hydrophobic and polar characteristics. PVPs are mostly used as vesicle transporters,[14,15] film formers,[16,17] binders,[18] and detoxifiers[19] in the pharmaceutical, cosmetic, food, and adhesive industries. Recently, PVP has been introduced on to cellulosic materials via NVP graft-copolymerization in order to combine the properties of PVP and cellulose. The resulting graft copolymers were shown to have improved metal sorption,[20] swelling,[21] thermal stability,[22] biodegradability resistance,[23] and solubility.[24]

The functionalization of HEC via its three available hydroxyl groups could lead to the development of novel materials with the properties of synthetic polymers but which are produced, in large part, from a renewable feedstock. The motivation of our work was the coupling of HEC with lactam groups in order to mimic the properties of PVP, leading to applications in new areas of advanced materials science for cellulosic materials. We aimed to lactam-functionalize HEC to give a unique bio-based polymer, rather than graft copolymerize NVP onto HEC. Our functionalization strategy is based on a low cost reaction centered on short reaction times under solvent-free conditions. Consequently, the lactam-functionalized HEC may compete favorably with a grafted copolymer equivalent from an industrial point of view. Like the graft copolymer, we hoped that our lactam-functionalized HEC might display significantly improved stability, swelling, solubility, and bacterial antiadhesive properties over unfunctionalized HEC. These enhancements, combined with straightforward synthesis, could provide a new type of cellulose hybrid material that could be produced economically on an industrial scale.

EXPERIMENTAL

Materials

All chemicals were purchased from Sigma Aldrich and were used as received. Solution state NMR spectroscopy was performed using either a Varian VNMR-700 spectrometer at 699.73 MHz (^{1}H) and 174.93 MHz (^{13}C), or a Bruker Advance 400 spectrometer at 400.13 MHz (^{1}H) and 100.60 MHz (^{13}C). For solid-state ^{13}C NMR spectroscopy, a Varian VNMRS spectrometer with a 9.4 T magnet was used at 100.56 MHz employing the cross-polarization method. Gel permeation chromatography was undertaken with DMF as solvent at 70 °C. 100 μL of solution agitated overnight to ensure complete dissolution was injected at a flow rate of 1.00 mL min^{-1} by a Viscoteck SEC autochanger model. A Viscoteck TDA 301 unit with triple detection (right angle laser scattering at 670 nm, differential refractometer, and viscometer) was used. Analyses were undertaken using OmniSEC 4.0 software. Elemental analyses (C, H, N) were performed using an Exeter CE-400 Elemental Analyzer. A Perkin Elmer Pyris 1 system was used for thermogravimetric analysis (TGA). A Brookfield Digital Viscometer model DV-I+ was used for measuring viscosities. A PowerWave^{TM} XS Microplate Spectrophotometer was used for measuring absorbance in 96-well microplates.

Synthesis and Characterization of Modified HECs

Functionalizing Agent: 1-(Hydroxymethyl)-2-Pyrrolidinone

The preparation of 1-(hydroxymethyl)-2-pyrrolidinone **2** followed a literature procedure[25]: In a one necked round bottom flask carrying a condenser, 2-pyrrolidinone **1** (10.62 g, 0.125 mol, 1 eq.) and potassium hydroxide (0.03 g, 0.534 mmol) were mixed and heated at 80 °C. Paraformaldehyde (3.78 g, 0.125 mol, 1 eq.) was added slowly and the mixture was stirred for 30 min at 80 °C. After the mixture was allowed to cool to room temperature, toluene was added, and the mixture was heated to 80 °C until complete dissolution occurred. The solution was allowed to cool to room temperature and filtered. The solid product was then recrystallized from toluene, filtered, and dried under vacuum at 40 °C overnight to afford 1-(hydroxymethyl)-2-pyrrolidinone (11.78 g, 82%) as a white powder; mp. 78–80 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H (ppm) 5.75 (s, 1H, CH$_2$—O**H**); 4.57 (s, 2H, C**H$_2$**—OH); 3.40 (*t*, $J = 7.2$ Hz, 2H, CH$_2$—C**H$_2$**—N); 2.22 (*t*, $J = 8.0$ Hz, 2H, C**H$_2$**—CO); 1.91 (app qn, $J = 7.6$ Hz, 4H, CH$_2$—C**H$_2$**—CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C (ppm) 174.2(**C**=O); 65.0(**C**H$_2$—OH); 45.1 (CH$_2$—**C**H$_2$—N); 31.1 (**C**H$_2$—CO); 17.6 (CH$_2$—**C**H$_2$—CH$_2$); EA (calculated): 51.95%C; 7.85%H; 11.97%N; EA (found): 51.99%C; 7.76%H; 11.94%N.

Functionalization of HEC with 1-(Hydroxymethyl)-2-Pyrrolidinone

The functionalization reaction was inspired by the modification of textiles with methylolated lactams.[26] In a three necked reactor fitted with an overhead stirrer, 2-hydroxyethylcellulose (HEC) **3** ($M_w = 250{,}000$ g/mol, DS = 1, MS = 2, 1.0 g, 4 mmol, 1 eq.), 1-(hydroxymethyl)−2-pyrrolidinone (HMP) **2** (5.0 g, 43 mmol, 10 eq.), and 2-amino-2-methyl-1-propanol hydrochloride (0.116 g, 0.923 mmol, 0.25 eq.) were mixed. The reactor was filled with nitrogen, closed to air, and the reaction mixture was heated at 155 °C for 30, 60, 90, 120, 300, or 1020 min. Once the mixture had cooled to room temperature, the product **4** was precipitated with approximately 200 mL acetone and collected by filtration. The recovered polymer was dissolved in water (10 mL) and dialyzed for 2 days through a 3500 Da MWCO membrane against milli-Q water. The precipitate was collected by filtration and dried under vacuum at 50 °C overnight to afford the modified HEC as a pale yellow powder in yields ranging from 50 to 70% based on the estimation of DS by ^{13}C NMR spectroscopy. Samples from each reaction were characterized by solid-state ^{13}C NMR spectroscopy using the cross-polarization method. Samples were also dissolved in DMSO-d_6 and were characterized using ^{1}H (700 MHz), ^{13}C (176 MHz), HMBC, and HSQC NMR experiments; see Table 1 for summaries of DS.

Evaluation of Physical Properties of HMP-Functionalized HEC

Assessment of Solubility

The solubilities of HEC and HMP-functionalized HEC ($DS_{\text{primary alcohol}} = 0.9$) were evaluated. Solvent (1 mL) (see Table 2) was added to cellulosic material (10 mg) in a vial. The vials were allowed to stand overnight at room temperature then the solutions were vortexed before determining visually the solubility.

TABLE 1 $DS_{primary\ alcohol}$ Determination for Different Reaction Times

Reaction Time (min)	$DS_{primary\ alcohol}$
30	0.1
60	0.9
90	0.9
120	0.9
300	0.8
1,020	0.3

Dye Release Study

HMP functionalized HEC ($DS_{primary\ alcohol} = 0.9$) and HEC were both tested for comparison purposes. Cellulosic material (0.1 g) and rhodamine B (3 mg) were ground to a powder using a pestle and mortar. The powder was then pressed at a force of 10 tonnes for 5 min using a KBr press, normally used for preparing IR samples. Each disc was placed in a vial with distilled water (20 mL). The samples were then left at 25 °C for 7 h, and the dye release from the discs was monitored visually, with photographs being taken every 10 min for the first hour and then every hour.

Measurement of Single Point Viscosity

Solutions of 3% w/w in water were prepared using HMP-functionalized-HEC ($DS_{primary\ alcohol} = 0.9$) and HEC. The dynamic viscosity of each solution was measured in mPa s using a Brookfield instrument which measured the force required for the torque to rotate in the immersed solution. In our experiment, the rotational speed of the torque and temperature were kept constant, that is, at 96 rpm and 25 °C respectively.

Assessment of Thermal Stability

The thermal stabilities of HMP-functionalized-HEC ($DS_{primary\ alcohol} = 0.9$) and HEC were compared using thermogravimetric analyses under nitrogen with a temperature ramp of 30 to 300 °C at a rate of 10 °C/min.

Bacteriological Studies

Escherichia coli K-12 wild-type strain [W3110, F^-, λ^-, *rpoS(Am), rph-1, Inv*(*rrnD-rrnE*)] and *Staphylococcus aureus* (3R7089 strain Oxford) were selected for bacteriological studies as representative Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) species. The following two experiments aimed to evaluate bacterial antiadhesion properties and monitor their viability and growth following exposure to materials treated with either HEC or HMP-functionalized HEC.

Antiadhesion Testing

Bacterial adhesion properties were assessed using the procedure of Wood et al.[27] Aqueous solutions containing 3% w/w of HEC and HMP-functionalized HEC were prepared. Squares (18 mm × 18 mm) of Whatman 3MM filter paper were submerged in each cellulosic solution and also in distilled water, as a control, for 40 min at room temperature. Each paper was then placed in a separate flask and dried overnight under vacuum at 50 °C. On each paper, bacterial culture (100 μL) grown to an A_{650nm} of 0.4 was applied. Sterile squares of polymer film (Saran wrap) were placed over each paper square to limit evaporation, then the squares were incubated at 37 °C for 24 h in a humid environment. The film was removed and 56/2 minimal salts buffer (150 μL) was added to each filter paper. Excess culture and the filter paper were placed in a 2 mL spin column vial and centrifuged to recover the bacterial culture. The resulting bacterial pellet was resuspended in Luria–Bertani (LB) broth and serial 10-fold dilutions from 10^{-1} to 10^{-5} were prepared using LB as diluent. Dilutions (10 μL) were spotted on to an LB agar plate, the plates were incubated for 18–20 h at 30 °C and the number of colonies was visually counted.

Bacterial Growth and Viability Testing

Aqueous solutions containing 0.04 g/mL of HEC and HMP-functionalized HEC were prepared. *E. coli* and *S. aureus* were grown to an A_{650nm} of approximately 0.25 in a 96-well microplate, then a solution (10 μL) containing either HEC or HMP-functionalized HEC was added. Appropriate controls using water (10 μL) or ampicillin solution (10 μL) were added to bacteria growing on the same plate. Bacterial growth at 37 °C was monitored at A_{650nm} as a function of time using a Biotek Synergy HT Multimode Microplate Reader.

RESULTS AND DISCUSSION

Synthesis

Our strategy for the preparation of lactam-modified HEC involves reaction with 1-(hydroxymethyl)-2-pyrrolidinone (HMP) **2**, following a procedure described in the patent literature.[26] HMP itself is synthesized from 2-pyrrolidone **1** and formaldehyde.

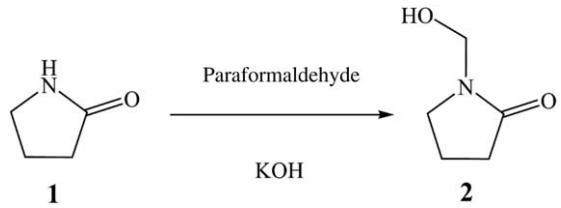
1-(Hydroxymethyl)-2-Pyrrolidinone

Paraformaldehyde was reacted with 2-pyrrolidone **1** in the presence of potassium hydroxide for 30 min at 80 °C

TABLE 2 Results of the Solubility Testing for HEC and HMP-Functionalized HEC (DS = 0.9)

Solvents	HEC	HMP-Functionalized HEC
Water	+	+
Dimethyl sulfoxide	+	+
Dimethylformamide	s	+
Cyclohexane	−	s
Dioxane	−	s
Butanone	−	−
Ethanol	−	s
Dichloromethane	−	+
Propan-2-ol	−	s
Chloroform	−	+
Toluene	s	s
Diethyl ether	−	−

s, swelling; +, soluble; −, insoluble.



SCHEME 1 Preparation of 1-(hydroxymethyl)-2-pyrrolidinone (HMP).

(Scheme 1) to obtain 1-(hydroxymethyl)-2-pyrrolidinone **2** in a yield of 82%.[25]

Functionalization of HEC Using 1-(Hydroxymethyl)-2-Pyrrolidinone

The functionalization reaction (Scheme 2) was performed in the absence of solvent. HMP is a liquid at the reaction temperature of 155 °C and served as both reaction medium and functionalizing agent. 2-Amino-2-methyl-1-propanol hydrochloride was used as a mildly acidic catalyst and a HMP:AGU unit ratio of 10:1 was chosen to provide a less viscous, and homogeneous reaction system. The mechanism of reaction is presumed to involve formation of an iminium ion from HMP under mild acid conditions (with loss of H_2O), followed by nucleophilic addition from a primary alcohol of HEC. A series of reactions were performed with varying reaction times of 30, 60, 90, 120, 300, and 1020 min to determine the influence of reaction time on the degree of functionalization of the primary alcohol group(s) of the HEC backbone, also named $DS_{\text{primary alcohol}}$.

The materials from each experiment were analyzed using solid-state ^{13}C and solution state ^{1}H and ^{13}C NMR spectroscopy. The spectra were similar for each reaction time point, and the assignment of signals for the products generated after a 90 min reaction time is considered below (Fig. 1). To confirm that the grafting process did not result in degradation of the cellulose chain, a weighed small portion of crude reaction product was dialyzed against a 50 kDa membrane and near quantitative mass recovery was obtained after lyophilization.

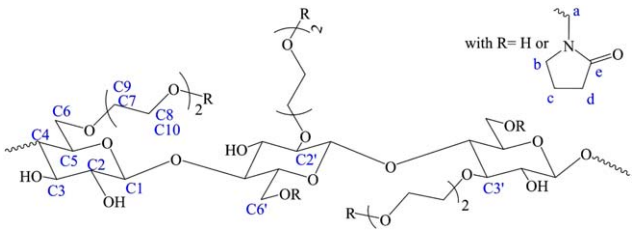
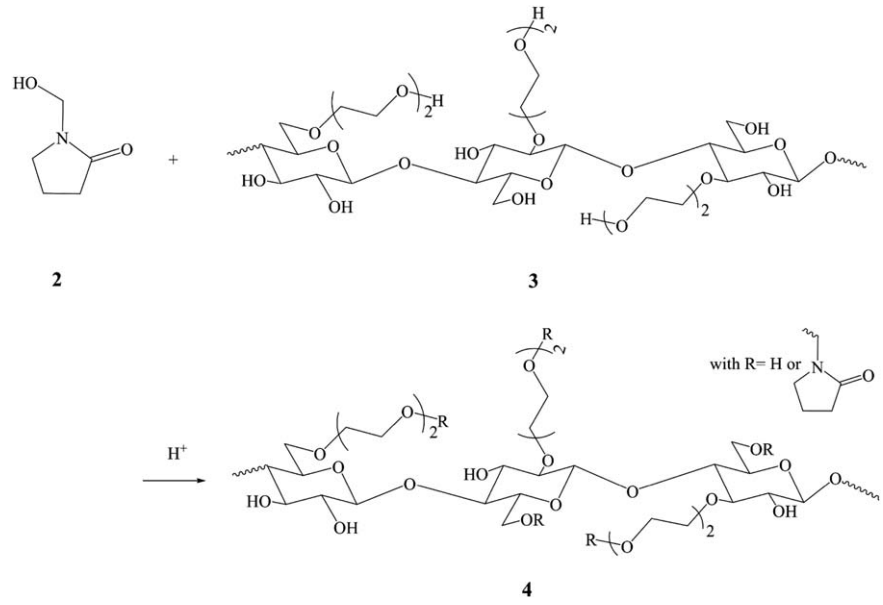


FIGURE 1 Numbering of the chemical structure of HMP-functionalized HEC. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The solid-state ^{13}C NMR spectrum [Fig. 2(b)] of HMP-functionalized HEC shows signals δ_C ~104, 83, 75, 71, and 62 ppm which are similar to those seen for HEC [Fig. 2(a)]. The signals δ_C ~104 and 83 ppm are respectively assigned to the anomeric carbon (C1) and C4. The signal δ_C ~75 ppm is assigned to the set of carbons, C2, C3, and C5. The signals δ_C ~71 and 73 ppm are assigned to the set of carbons from C6 (R≠H) to C9 representing the ethylene oxide side chains and the signal δ_C ~62 ppm corresponds to the carbons C6 (R═H) and C10 (R═H). However, a set of signals δ_C ~19, 32, 47, and 177 ppm is detected in the spectrum of HMP-functionalized HEC [Fig. 2(b)] but not in HEC [Fig. 2(a)]. These signals are assigned to the pyrrolidone ring of HMP and the strong decrease of the signal δ_C ~62 ppm indicates the modification of the primary alcohol(s) of HEC by HMP. The broadening of the peaks at δ_C ~71 and 73 ppm (the



SCHEME 2 HEC 3 functionalization reaction with HMP 2.

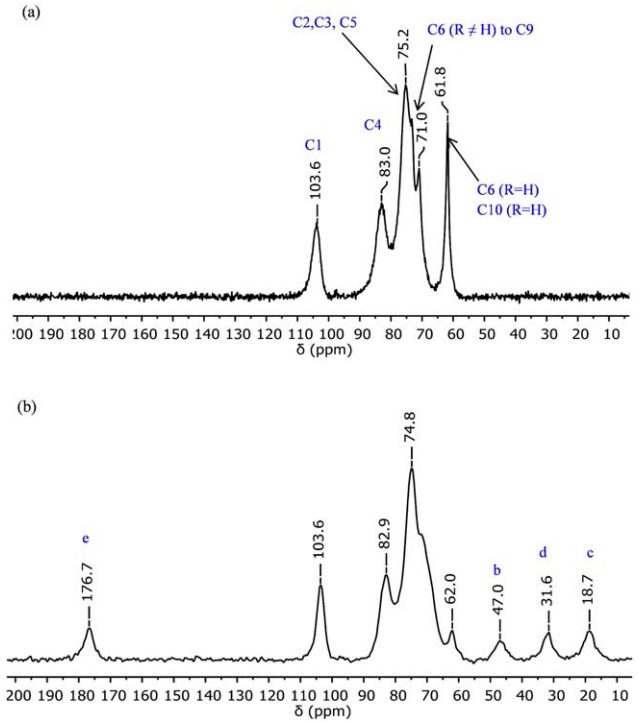


FIGURE 2 Solid-state MAS CP ^{13}C NMR spectra of (a) HEC and (b) HMP-functionalized HEC ($DS_{primary\ alcohol} = 0.9$) using cross-polarization (CP) method. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

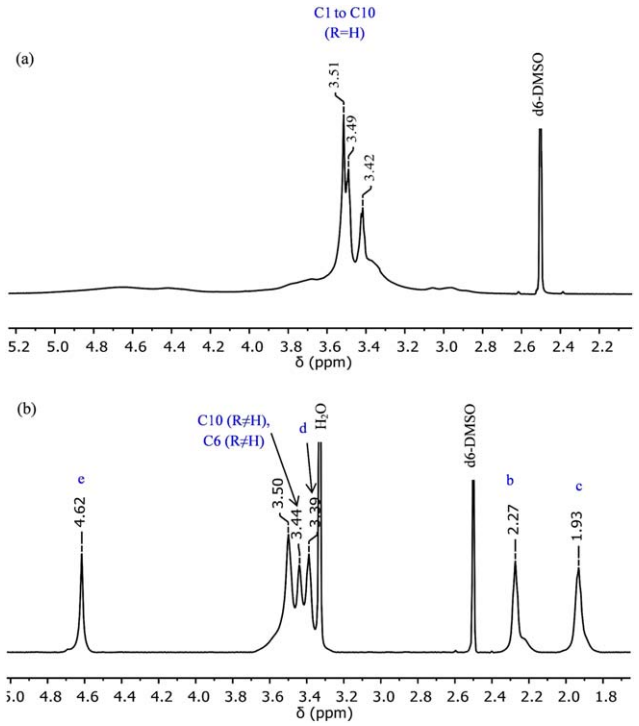


FIGURE 3 ^{1}H NMR spectrum (700 MHz, d6-DMSO) of (a) HEC and (b) HMP-functionalized HEC ($DS_{primary\ alcohol} = 0.9$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

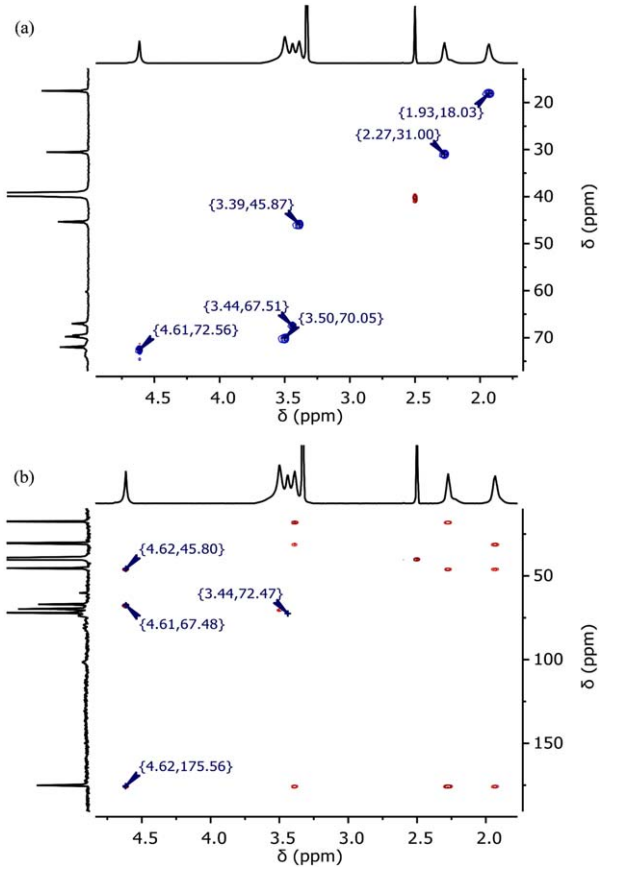


FIGURE 4 ^{1}H-^{13}C NMR correlation spectra of the HMP-functionalized HEC (a) HSQC (b) HMBC. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

latter coalesces with the peak at δ_C ~75 ppm) suggests the appearance of a new, overlapping peak in this range which can be assigned to HMP coupled to the side chain of HEC. In addition, the —$\mathbf{C}H_2O$— signal of HMP is most likely obscured by these signals.

The solution state ^{1}H NMR spectrum of the functionalized HEC [Fig. 3(b)] shows signals δ_H ~1.93, 2.27, and 3.39 ppm that correspond to the pyrrolidone ring and the signals δ_H ~3.44 and ~3.50 ppm are in line with those expected for an HEC derivative [Fig. 3(a)]. Most importantly, however, the signal δ_H ~4.62 ppm appears to be consistent with the pendent —C$\mathbf{H_2}$— group of HMP attached to a hydroxyl group of HEC. The chemical shift, although similar to, functionalizing agent HMP, is distinct from that of HMP (δ_H ~4.57 ppm), which supports the idea of the OH of HMP being exchanged with an *O*-alkyl fragment from HEC. The broad signals across the baseline are assigned to the rigid cellulose backbone whereas the sharper signals are due to the more mobile HMP side groups.

In order to demonstrate the attachment of HMP to HEC, HMBC and HSQC solution NMR experiments were performed (Fig. 4). The HSQC experiment [Fig. 4(a)] permits the

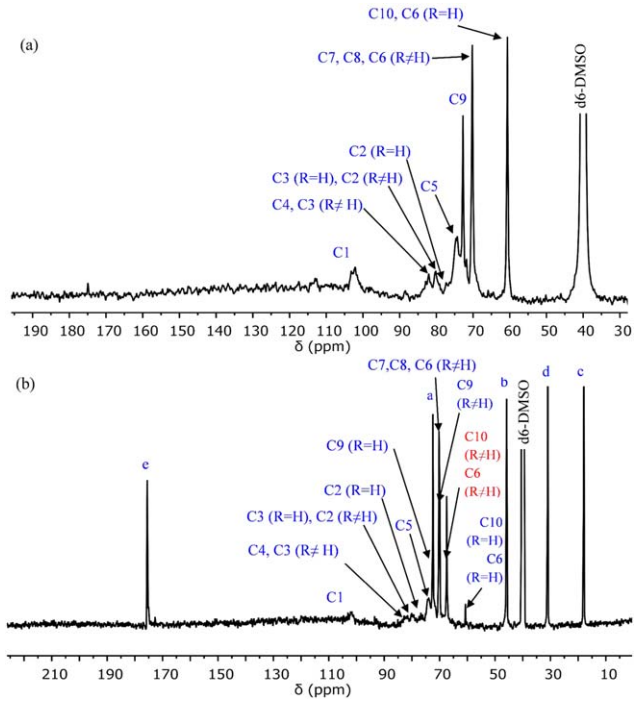


FIGURE 5 ^{13}C liquid state NMR (DMSO-d6, 176 MHz) spectrum of (a) HEC and (b) HMP-functionalized HEC ($DS_{primary\ alcohol} = 0.9$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

assignment of the solution state ^{13}C NMR spectrum [Fig. 5(b)]. Critically, a signal consistent with the pendent —$\mathbf{C}H_2$— group of HMP lies at δ_C ~72.6 ppm. Signals between δ_C ~60 ppm to δ_C ~70 ppm correlate to the HEC backbone and the signals δ_C ~18, 31, and 46 ppm correspond to the pyrrolidinone group, which further supports the presence of both HEC- and HMP-like species within the isolated material.[28,29]

The HMBC experiment [Fig. 4(b)] highlights the covalent bonding between HMP and HEC. The δ_H ~4.62 ppm signal, which is assigned to the pendent —$\mathbf{CH_2}$— group of HMP, correlates with δ_C ~45.8, 67.5, and 175.5 ppm. The signals δ_C ~45.8 and 175.5 ppm are respectively assigned to —$\mathbf{C}H_2$—N— and the carbonyl —$\mathbf{C}$=O group of the pyrrolidinone. The presence of correlation between δ_C ~67.5 ppm and δ_H ~4.62 ppm suggests covalent bonding between the pendent —CH_2— group of HMP and the primary alcohol group(s) of HEC. This is consistent with the primary C6 (R=H)/C10-methylene-hydroxyl group being the most nucleophilic hydroxyl on HEC, in agreement with the established order of hydroxyl group nucleophilicities of HEC (C10 ≥ C6(R=H) >> C2 > C3).[30] Furthermore, the low peak intensity for δ_C ~60.6 ppm, which corresponded to the free, unreacted primary alcohol of unfunctionalized HEC, shows that the functionalization proceeds to near completion, exclusively at the C10 and C6 (R=H) positions on the HEC backbone. The signal δ_C ~72.5 ppm of the pendent —$\mathbf{C}H_2$— group of HMP correlates to δ_H ~3.44 ppm. The δ_H ~3.44 ppm can be assigned to the protons at the C10 and C6 position where R≠H which serves to provide a second confirmation of the ligation between HMP and HEC. For reference purposes, the solution state ^{13}C NMR spectrum of HEC is shown in Figure 5(a).

The ^{13}C NMR assignment of the functionalized HEC [Fig. 5(b)] also permits quantification of functionalization through integration of methylene signals, which are expected to relax at similar rates. The signals δ_C ~60.6 and 67.5 ppm have been assigned to the carbons at C6/C10 positions where R=H and R≠H, respectively. Using the relative integrals of these C6/C10 signals, the $DS_{primary\ alcohol}$ values for the materials from each functionalization reaction were estimated (Table 1).

For a reaction time of 30 min, the calculated $DS_{primary\ alcohol}$ was 0.1. This low value is explained by the heterogeneous nature of the reaction mixture. HMP was used as the reaction medium and its incomplete melting after 30 min created a heterogeneous system. A $DS_{primary\ alcohol}$ value of 0.9 was calculated for reaction times of 60, 90, and 120 min, showing that the functionalization reaction of the primary alcohols was quasi-complete. However, a $DS_{primary\ alcohol}$ value less than 1 indicates either limited accessibility of HMP to the HEC backbone because of H-bonding in the HEC, or an incomplete equilibrium process. For a reaction time of 300 min, a decreased $DS_{primary\ alcohol}$ value of 0.8 was determined and a $DS_{primary\ alcohol}$ of 0.3 was estimated after 1020 min. A possible reason for this reduction is the reversibility of the functionalization reaction. The functionalized HEC material can potentially liberate HMP which could decompose into formaldehyde and 2-pyrrolidone. This decomposition reaction is essentially irreversible given that formaldehyde can be lost as a gas from the system.

Evaluation of Physical Properties

Solubility

The solubility of the HMP-functionalized HEC ($DS_{primary\ alcohol} = 0.9$) was tested and compared with the unfunctionalized material, HEC. A concentration of 10 mg/mL of cellulosic material was chosen. The results of the tests are summarized in Table 2. HEC, which is not soluble in organic solvents, became soluble or swelled in these same organic solvents such as DMF, DCM, and EtOH after functionalization with HMP. HMP contains a lactam group, which is also present in poly(vinylpyrrolidone) (PVP). PVP is well-known for its solubility in both aqueous and organic solvents due to its amphiphilic character. The introduction of lactam groups on to HEC could therefore lead to the development of an amphiphilic derivative of HEC which could see its use extended for industrial applications.

Dye Release Study

HEC is used in the preparation of pharmaceutical tablets as a binder because of its biocompatibility and biodegradability, however, the modification of HEC with HMP could affect the release profile of the active molecule from such a tablet. Therefore, the dye release test was undertaken to simulate the release of a drug from a tablet prepared from HEC and

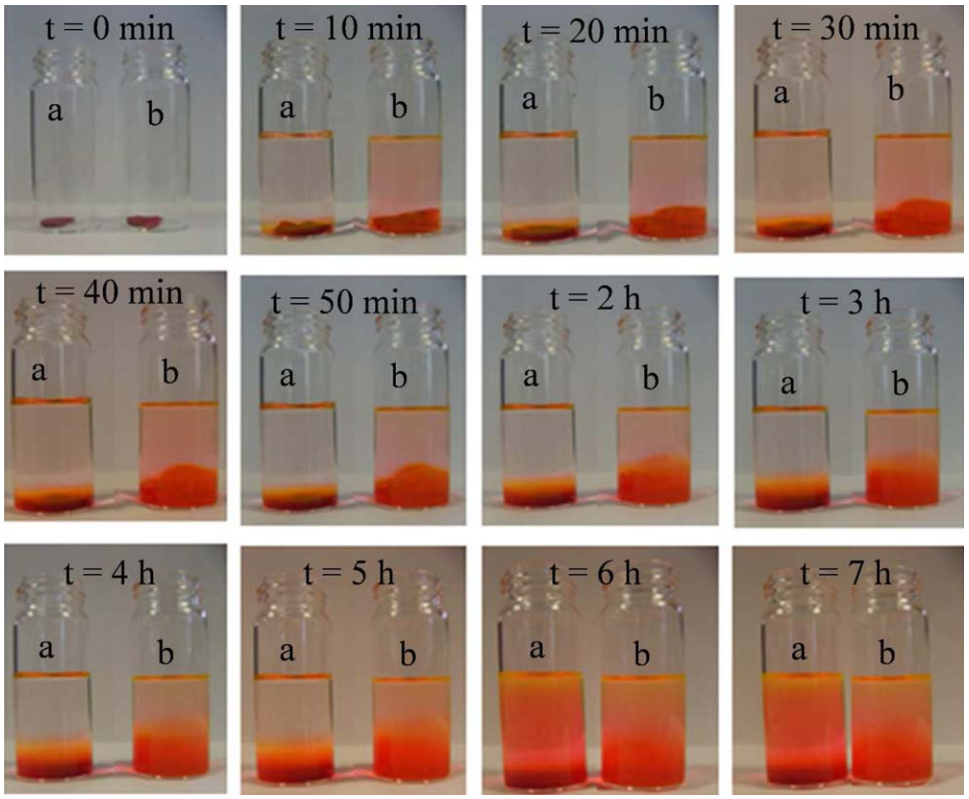


FIGURE 6 Results of the swelling test of (a) HEC (b) HMP functionalized HEC (DS = 0.9) using rhodamine B. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

HMP-functionalized HEC ($DS_{primary\ alcohol} = 0.9$). A water soluble dye, rhodamine B was used as a model drug and tablets were prepared using a KBr press. The dye release was evaluated qualitatively as a function of time and is shown in Figure 6.

The dye contained in the HEC disc was released into water, indicating the beginning of the disintegration of the disc. The dye diffusion started from the bottom of the vial where the HEC disc was located, leaving the top of the vial clear. The diffusion of the dye increased slightly during the first hour. Over the following hours, an increase of the dye diffusion was continuously observed with disc disintegration. After 6 h, the dye was present throughout the aqueous solution and the disc had completely disintegrated. However, the color effect suggests a gradual dye concentration decrease from the bottom to the top of vial. A homogeneous concentration in the vial may be achievable with a longer experiment time or stirring.

Regarding HMP functionalized HEC, the disc swelled continuously with time in water. The dye contained in the centre of the disc was not released, highlighting the swelling of the disc, however, the dye at the surface was released and diffused completely into the water. The swelling of the disc increased rapidly during the first hour without dye release into the water. After 2 h, the gel-like disc started to release the dye, and, over the following hours, the swollen disc decomposed. The dye was then released and diffused through the water. At 6 h, the observations were similar to the HEC disc with a gradual change in dye concentration through the solution.

HMP functionalization clearly affected the dye release properties of HEC. The introduction of HMP to HEC was expected to interrupt the H-bonding network, resulting in increased water solubility. Furthermore, the polarity of the amide group of HMP may further improve the solubility of functionalized HEC in water as well as in polar organic solvents, compared with HEC. However, HMP presents also a partially hydrophobic character due to the presence of the lactam ring. The bonding of HMP to HEC may cause an increase of HEC hydrophobicity (estimated log P of *N*-methyl-pyrrolidone ranges from -0.38 to -0.57[31]). The hydrophobic/hydrophilic properties could explain the tendency of the functionalized HEC to swell in water during the first hour with subsequent dissolution.

Thermal Stability

The thermal stability was studied to determine the temperature range over which HMP-functionalized HEC can be processed and/or used without any degradation. The weight loss on heating HMP-functionalized HEC ($DS_{primary\ alcohol} = 0.9$) and HEC as a function of temperature are recorded in Figure 7. HEC [Fig. 7(a)] did not lose weight from 10 to 200 °C indicating its thermal stability over this temperature range.

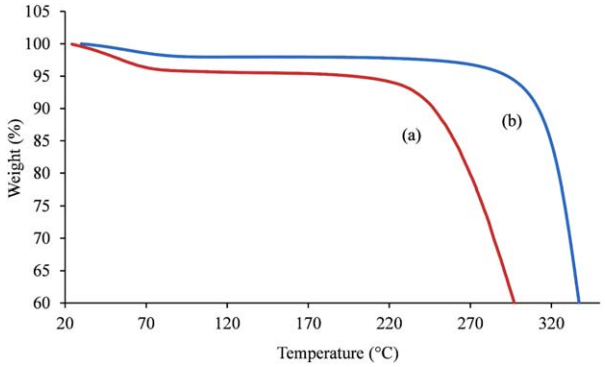


FIGURE 7 Evolution of weight loss as a function of temperature by TGA (a) HEC; (b) HMP-functionalized HEC ($DS_{primary\ alcohol} = 0.9$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

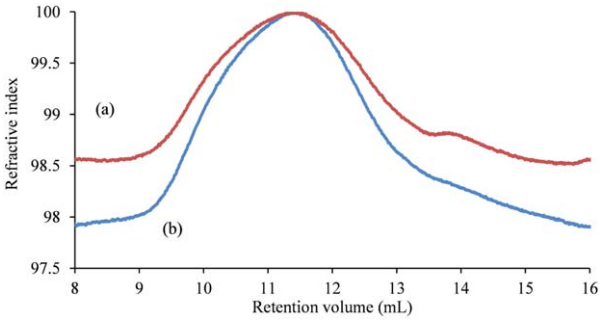


FIGURE 8 SEC analysis of (a) HEC and (b) HEC treated at 155 °C overnight in presence of 2-amino-2-methyl-1-propanol hydrochloride. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

However, weight loss increased sharply above 210 °C indicating the beginning of HEC decomposition. At 300 °C, a weight loss of 40% was measured. Regarding the functionalized HEC [Fig. 7(b)], its weight was relatively stable over the temperature range 10 °C to 210 °C. From 250 °C, the functionalized HEC started to show a very slight weight loss, however, at 300 °C, the functionalized HEC had lost only ~4% of its weight.

The measured weight loss at 300 °C of the functionalized HEC was ten times less than that of the HEC material. At a significantly lower temperature, the functionalization of HEC with HMP therefore generated a more thermally stable polymer compared with unfunctionalized HEC due to the increased molecular weight of HMP-functionalized HEC compared with unmodified HEC. This results in a broader temperature range over which HMP-functionalized HEC can be used and/or processed for desirable applications.

Evaluation of Integrity of HEC Backbone Following Chemical Modification

The breadth of the HEC backbone solution state NMR signals (Fig. 3) is a strong indication of the high molecular weight of HMP-reacted HEC, nonetheless further analysis was performed to probe the integrity of the cellulose backbone following reaction with HMP. GPC analysis was performed before and after heating HEC overnight with the catalyst 2-amino-2-methyl-1-propanol hydrochloride at 155 °C in *N*-methyl-pyrrolidone (20 mL). Both GPC traces (Fig. 8) were found to overlay, indicating little or no change to the HEC backbone. Furthermore, HEC reacted with HMP was dialyzed against a 50 kDa membrane and near quantitative mass recovery was obtained after lyophilization. Thus we conclude that reaction with HMP did not cause degradation of the cellulose backbone.

Viscosity Measurement

The measured dynamic viscosities for HEC and functionalized HEC were respectively 453.1 and 15.6 mPa s. The viscosity of functionalized HEC is approximately 30 times lower compared with that for HEC. The introduction of the lactam group into HEC causes both the breaking of the H-bonding network and an increase in the distance between HEC chains. As a consequence, the water accessibility into the structure is improved, the solubilization of functionalized HEC is enhanced, and this, in turn, causes the drastic viscosity reduction.

Bacteriological Testing

Cellulosic materials are widely used in regenerative tissue culture, drug delivery, personal care, cellular immobilization, and wound healing applications.[32,33] The adherence of bacteria to such surfaces can lead to the establishment of infections and the formation of complex biofilms that hinder treatment. A plausible route to reduce the risk of infections is to chemically modify material surfaces, where the modified surfaces could result in the death and/or the reduced adhesion of bacteria. For instance, the presence of cationic molecules such as imidazolium groups is known to kill bacteria.[34] In 2007, Cheng et al.[35] investigated the antiadhesion properties of bacteria of zwitterionic surfaces. The reduction in bacterial adhesion was most evident with zwiterrionic and hydrophilic/neutral polymers, with both Gram-positive and Gram-negative bacteria behaving in a similar fashion. It was anticipated that the introduction of lactam groups would confer dipolar properties to HEC and thus could enhance its antiadhesion properties against bacteria. The influence of the HMP-functionalization of HEC on bacterial viability, growth and adhesion was therefore examined using representative Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) strains to investigate bacterial selectivity.

For the viability and growth experiments, a solution in water of HEC and HMP-functionalized HEC was added to a 96-well microplate containing bacteria grown to an A_{650nm} of approximately 0.25. Growth was recorded as a function of time for both *E. coli* and *S. aureus* (Fig. 9). For antiadhesion testing, filter paper was impregnated with a solution containing either HEC or HMP-functionalized HEC; sterile water was used as a control. Cultures of *E. coli* and *S. aureus* in mid-logarithmic phase (A_{650nm} 0.4) were applied to the surface of the paper and the adhered bacteria recovered after 24 h at 37 °C. Serial tenfold dilutions of the recovered bacteria

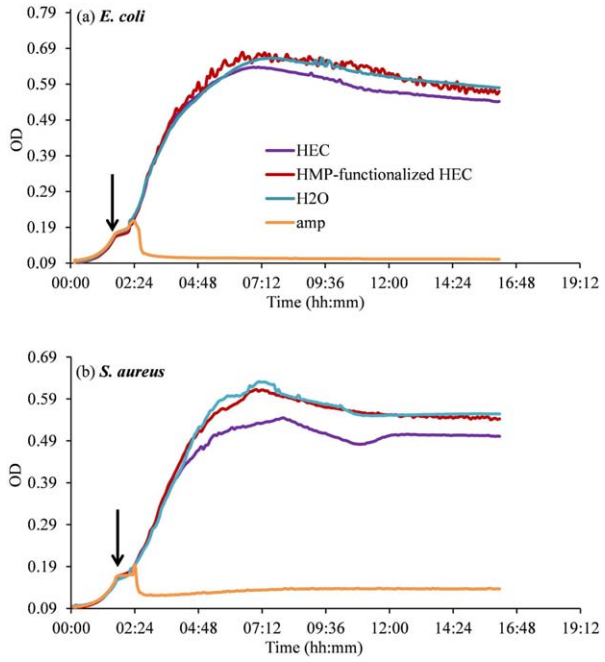


FIGURE 9 Growth of *E. coli* and *S. aureus* as a function of time after addition (indicated by arrows) of solutions containing HEC, HMP-functionalized HEC ($DS_{primary\ alcohol} = 0.9$), water ($H_2O$) and ampicillin (amp). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

were prepared and spotted on to an agar plate (Fig. 10). The plate was incubated and bacterial recovery was evaluated visually by counting colonies at appropriate dilutions (Table 3).

For *E. coli*, the growth and viability (as judged by optical density) of the bacteria [Fig. 9(a)] were largely unaffected by the presence of HEC and HMP-functionalized HEC solutions indicating that these materials lack bactericidal properties. Regarding the antiadhesion properties [Fig. 10(i) and Table 3), the number of recovered bacteria from the filter paper treated with water ([Fig. 10(i)(b)] was slightly lower (1.0×10^7 cfu/mL) than that coated with functionalized HEC (1.36×10^7 cfu/mL), whereas a significantly larger number of bacteria were recovered from samples treated with HEC (1.96×10^8 cfu/mL). *E. coli* therefore adhered most strongly to the control filter paper (treated with water). HEC treatment [Fig. 10(i)(c)] significantly enhanced the bacterial antiadhesion properties of the paper with a 20-fold increase in the recovery of bacteria. It also enhanced the growth of bacteria with 2× more bacteria recovered compared with the control (7.2×10^{-7} CFU/mL), which is the number of deposited bacteria on the surface. The functionalized HEC-treated paper [Fig. 10(i)(d)] showed considerably reduced antiadhesion properties compared with HEC, with only a 1.4-fold increase in the number of bacteria recovered relative to the paper treated with water.

For *S. aureus,* the viability and growth [Fig. 9(b)] was unaffected following addition of a solution containing HMP-

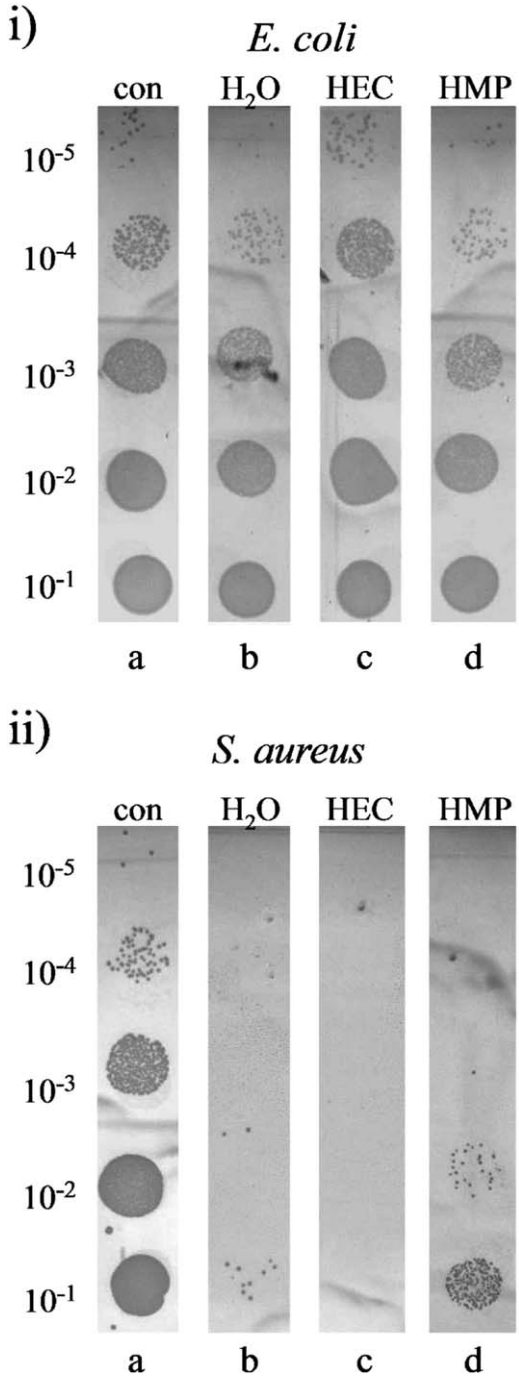


FIGURE 10 Adhesion testing of (i) *E. coli*, (ii) *S. aureus* monitoring the recovery of viable bacteria from filter paper pretreated with (b) H_2O; (c) HEC and (d) HMP functionalized HEC ($DS_{primary\ alcohol} = 0.9$). A control sample (a) not exposed to filter paper was also tested.

functionalized HEC. HEC did reduce the bacterial density as cells entered the stationary phase of growth compared with HMP-functionalized HEC, although the results are similar to those seen with *E. coli* [Fig. 9(a)]. In antiadhesion experiments [Fig. 10(ii) and Table 3], the number of recovered bacteria from the paper treated with the solution of HMP-

TABLE 3 Recovery of Bacteria from Filter Papers Treated with Water, HEC, and HMP-Functionalized HEC

		Cells/mL	SD	Fraction Recovered
E. coli	Control	7.2×10^7	1.6×10^7	1.0
	H_2O	1.0×10^7	4.0×10^5	0.14
	HEC	1.4×10^8	1.2×10^7	1.9
	HMP	1.9×10^7	0	0.27
S. aureus	Control	2.4×10^8	8.0×10^7	1.0
	H_2O	5.4×10^3	1.4×10^3	2.2×10^{-5}
	HEC	0	0	0
	HMP	8.3×10^4	1.0×10^3	3.5×10^{-4}

functionalized HEC [Fig. 10(ii(d)] was the highest (8.3×10^4 cfu/mL) compared with those treated with water (5.4×10^3 cfu/mL) or with the HEC solution (0 cfu/mL). For the paper treated with HEC [Fig. 10(ii)(c)], bacteria were not recovered indicating complete adhesion of *S. aureus* to the paper since HEC does not show any significant bactericidal effects [Fig. 9(b)]. In the case of *S. aureus*, the chemical modification of HEC with HMP clearly improved the antiadhesion properties relative to the unfunctionalized HEC.

In summary, the antiadhesion testing reveals a marked difference in the behavior of HEC and HMP-functionalized HEC between the two bacterial species. This difference may be due to altered hydrophobicity, one of the criteria influencing bacterial adhesion. In fact, bacterial adhesion depends on surface properties, including roughness, charge, hydrophobicity, and chemical composition, the hydrophobicity and charge of the bacterial envelope and other surface structures.[36] Bacteria with a hydrophobic exterior will adhere preferentially to hydrophobic substrates, whereas bacteria with hydrophilic surfaces favor hydrophilic materials.[37] The *E. coli* bacteria used in this study present a predominantly negatively charged, hydrophilic surface composed of lipopolysaccharides and outer membrane proteins, whereas *S. aureus*, although possessing a negatively-charged surface composed of peptidoglycan, techoic acids, and surface proteins, is radically different in character.[38] The filter paper treated with water presents a more hydrophilic surface than that treated with HEC, hence the adhesion of *E. coli* increases slightly, whereas almost all of the *S. aureus* cells were retained. Treatment with a solution containing HEC may increase the hydrophobicity of the paper, reducing bacterial adhesion by *E. coli* and increasing adhesion by *S. aureus*. Introduction of lactam groups onto HEC decreases its hydrophobic character and improves bacterial adhesion by *E. coli* but significantly decreases bacterial adhesion by *S. aureus*, reflecting the differences in the cell envelope architecture in these bacteria.

CONCLUSIONS

Our strategy to introduce lactam groups onto a cellulosic material via the functionalization of HEC with HMP was found to be highly efficient. The synthesis of the functionalizing agent, HMP **1**, gave reproducibly high yields. The modification of HEC with HMP gave a maximum $DS_{\text{primary alcohol}}$ of 0.9. The incomplete functionalization of the primary alcohols of HEC is consistent with the particular structure of the cellulose, notably the presence of intramolecular and intermolecular hydrogen bonding which blocks the accessibility of the primary alcohols to the functionalizing agent. The conditions of the modification reaction with HMP have been shown not to lead to any significant degradation of the cellulose backbone. The lactam-functionalized HEC swells in water whereas the HEC dissolves. Moreover, the viscosity of the lactam-functionalized HEC decreased while the thermal stability increased compared with unmodified HEC. Furthermore, bacteriological assays revealed an increase in bacterial adhesion by *E. coli* and, reciprocally, a decrease in adhesion of *S. aureus* to lactam-functionalized HEC in comparison to unfunctionalized HEC. This can be explained by both the breakage of the hydrogen bonding network and the introduction of the lactam groups which enhance the material's hydrophilicity and has contrasting effects depending on the nature of the bacterial envelope surface. The HMP-functionalized HEC could be used as a controlled drug delivery system and as a coating which is thermally more stable than the parent HEC and can prevent the adhesion of gram + bacteria. Overall, the modification of HEC did not lead to new applications but added a considerable value to the use of a cellulosic material in industry because of the alteration of its properties (solubility, viscosity, and thermal stability).

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