Antimicrobial films based on cellulose-derived hydrocolloids. A synergetic effect of host–guest interactions on quality and functionality

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A series of active films based on biodegradable cellulose-derived hydrocolloids capable of controlled release of antimicrobial propionic acid (PA) was prepared. β-Cyclodextrin (β-CD), usually used for encapsulation of lipophilic compounds, was utilized in this research to host the hydrophilic PA. It was found that addition of β-CD to the film forming solutions notably enhanced the hydrocolloid matrix capacity and resulted in up to a ten-fold increase in the amount of loaded PA. In addition, β-CD resulted in a two-fold prolongation of the effective PA release duration. β-CD alone caused undesired effects on the physical, mechanical and morphological properties of the hydrocolloid films. Interestingly, when β-CD was combined with PA in the film formulation, its undesired effects were significantly subdued. The antifungal activity of the films was demonstrated on fresh harvested wheat grains. Films containing β-CD and PA were found to be effective in preventing fungal growth on wheat grains. Thus, incorporation of β-CD and PA in hydrocolloids matrices demonstrated a synergetic effect and resulted in the formation of biodegradable active films that benefit good physical and mechanical properties, high active agent content, prolonged release ability and effective antimicrobial properties.

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1. Introduction

Bio-application of natural hydrocolloids-based active films for storage and controlled release of antimicrobial agents is of current research interest [1–3]. Such systems are of high relevance for pharmaceutical and food industries [4–7]. Cellulose derivatives are renewable, widely available eco-friendly hydrocolloids that have a wide range of applications such as packaging, tissue engineering, wound dressing, and nano-composite materials [8–11]. Cellulose derived materials may also be utilized as biodegradable systems for controlled release of antimicrobial agents in particular [12–14]. As recent developments in this field are gaining popularity, more and more edible films are being explored for their potential to be a useful tool to protect food produce from spoilage [15,16]. Further intended applications include food packaging to aid preservation and lengthen shelf-life.

One of the successful active agents with natural antimicrobial properties is propionic acid (PA). PA is a powerful generally recognized as safe antimicrobial agent [17]. PA is used in the food industry for fungal and yeast spoilage inhibition [18,19]. However, its volatile nature leads to undesired drawbacks such as necessity in multiple treatments, lack of balanced dispersion, corrosion of equipment, and in some cases aftertaste in the food products [20]. Systems for controlled release of PA are therefore much preferred.

Encapsulation systems may help reduce natural volatility and allow controlled release of active agents, improving their effectiveness [21]. Cyclodextrins (CDs) are key components in a large number of encapsulation systems [22,23]. β-Cyclodextrin (β-CD), much like the rest of the CD family, is a cyclic oligosaccharide with a hydrophobic cavity and an outer hydrophilic surface and is widely used to encapsulate lipophilic compounds [24,25]. However, interactions of β-CD with hydrophilic guest molecules remained out of scientific literary focus and are scarcely explored [26].

In this research we aim to develop antimicrobial active films based on biodegradable cellulose-derived hydrocolloids. Three cellulose derivatives were chosen as raw materials: carboxymethylcellulose (CMC), hydroxypropyl methylcellulose
(HPMC) and methylcellulose (MC). PA was chosen as the active ingredient due to its natural antimicrobial properties. β-CD was engaged to increase PA loading capacity in the active films. It was assumed that β-CD’s multiple hydroxyl groups will be sufficient for the necessary hydrogen bonds between it and PA and may allow effective hosting and a more prolonged release of PA. CMC, HPMC and MC-based films were uploaded with various concentrations of PA and β-CD. β-CD’s influence on the films’ loading capacity and PA release rate was studied. The antimicrobial activity of the prepared films was tested. In addition, mechanical (tensile stress, Percent elongation at break, Young’s modulus), physical (water vapor transmission rate, thermal stability and degradation) and morphological properties of the prepared films were studied.

2. Experimental

2.1. Materials

All reagents were of analytical grade and used without further purification. CMC (sodium salt), HPMC, MC and PA were purchased from Alfa Aesar (Heysham, England). β-CD was purchased from Chem-Impex Int’l Inc. (Wool Dale, IL, USA).

2.2. Preparation of active films and loading capacity studies

For each cellulose type (CMC, HPMC and MC) the following series of film forming solutions (FFS) were examined. Three series with different concentrations of β-CD (0, 2.5 and 5% w/w) were prepared. Each set included 8 types of FFS which varied in PA concentration (0.2, 0.3, 1.6, 4.8, 9.7, 14.6, 19.4 or 29.1% w/w). PA was dissolved in 50 mL of double distilled water (DDW), then β-CD was added and the reaction was heated to 50 °C for 1 h. Next, a cellulose polymer was added (2% w/w) and the reaction was stirred for 2 h at 50 °C (MC’s FFS were not heated and stirred at 23 °C for 2 h). All films were obtained by pouring 5 mL portions of the FFS into Teflon Petri dishes (5 cm in diameter) and dried at 23 °C overnight at relative humidity (RH) of 65 ± 2%. The prepared films were stored at −18 °C.

Inspected film samples were tested for PA contents by extracting them for 2 h in 30 mL DDW before titrations. This allowed for the cellulose matrix to break down and release PA present in the film. Acid-base titrations were then performed with sodium hydroxide (0.1 M) as the titrant and phenolphthalein as a pH indicator. All titrations were performed in triplicates. The different cellulose types’ and β-CD’s contribution to the titration results were taken into account and subtracted. The weight β-CD introduced into the films was accounted for using Eq. (1). This equation shows the correction factor used to determine the effect of β-CD on the films’ mass.

\[
P_{A_{\text{conc.}}} [\% \text{w/w}] = \frac{m_{PA}}{m_{\text{film}} \cdot (m_{\text{cellulose}} / (m_{\text{cellulose}} + m_{\beta-CD}))} \times 100\% \quad (1)
\]

Final PA concentration results were attributed in relation to the cellulose mass alone. This allowed viewing the full effect of β-CD’s loading capacity when comparing these results to films without β-CD at all.

2.3. Long range release studies

Films based on cellulose derivatives (CMC, HPMC or MC) were prepared utilizing 9.7% w/w of PA and 0.25 or 5% w/w of β-CD according to the previously described method. Films were kept in a controlled atmosphere room at a steady 20 °C at RH 65% and periodically sampled for PA content via acid-base titrations.

2.4. Film thickness

Film thickness was determined by averaging six measurements at different points for three films of each type using a desktop Mitutoyo digital thickness gauge with an accuracy of ±0.001 mm (Mitutoyo Corp, Kawasaki, Kanagawa, Japan).

2.5. Films’ water vapor transmission rate (WVTR)

WVTR was measured gravimetrically using the ASTM E-96 method [27] adapted to hydrophilic films [28]. Briefly, vessels filled with 10 mL of DDW were sealed with films using carbon tape, then weighed and placed in desiccators containing dry silica gel (50% RH; 23 °C). The vessels were allowed to stand for 2 h to reach an initial equilibrium, and then weighed with 8 h intervals during 48 h. Water vapors escaped by permeation through the film and the water loss was recorded by analytic balance (±0.0001 g). Water vapor transmission rates (WVTR g/m²h) were obtained by plotting the weight loss vs time in a linear regression \((r > 0.99)\) and dividing the slope by the exposed film area \((m²)\).

2.6. Films’ thermal analyses

Thermal stability and degradation of films were analyzed by thermal gravimetric analysis (TGA) and differential thermal analysis (DTA). Tests were performed with a TGA-Mettler apparatus (TG-50 DSS50 model, Mettler-Toledo, OH, USA) with temperature profile settings being 25–550 °C at 10 °C/min under N₂. The resulting graphs were normalized for comparative purposes.

2.7. Tensile stress (TS), percent elongation at break (PE) and Young’s modulus (YM)

TS, PE and YM were determined using an Instron 3345 instrument with an Instron force transducer load cell (Norwood, MA, USA). Tests were performed at a speed of 1 mm/s. TS was expressed in [MPa] and was calculated by dividing the maximum load [N] by the cross-sectional area [m²]. PE was calculated by dividing the extension at the moment of rupture by the initial gauge length of the samples and multiplying by 100. YM was expressed in [MPa] and was determined by the ratio of the stress along an axis over the strain along that axis in the range of stress. All measurements were performed in triplicate for each film type.

2.8. Microscopy

Film samples were inspected using a DS-F1i digital camera mounted on a Leica MZFLIII binocular microscope. Environmental scanning electron microscope (ESEM) images were recorded using a model Quanta 200FEG (Field emission gun) Schottky field emitter of FEI with an ETD detector.

2.9. Antimicrobial activity of the films against post-harvest storage of wheat grains

Post-harvest non contaminated wheat grains (at moisture content of 12%) were exposed to films. To sterile 500 mL beakers, 5 g of grains were added. The prepared films were added to the beakers so that they surrounded the grains from all sides. Different types of treatments were examined; (a) control (grains only), (b) films with β-CD, (c) films without β-CD, (d) pure PA. All of the treatments were tested in triplicate. Each beaker was sealed and placed at rt for 30 days. Following that period, the beakers were opened and the grains were taken from infestation evaluation using the direct plating method (surface sterilization by 2 min in a 2% solution of...
sodium hypochlorite, followed by 2 min rinsing in sterile water and then plating on potato dextrose agar containing 0.005% of chloramphenicol in Petri dishes – ten grains per plate). The number of infested grains was counted daily during 14 days of incubation.

2.10. Statistical analysis

Microsoft Office Excel spreadsheets were used to calculate means, standard deviations and 95% t confidence intervals. The statistical analyses were carried out using JMP statistical software program, version 7 (SAS Institute Inc., Cary, NC, USA) including a one-way analysis of variance (ANOVA) followed by the Tukey–Kramer honestly significant difference (HSD) post hoc test. Results marked with different letters are significantly different at \( p \leq 0.05 \).

3. Results and discussion

3.1. Loading and release capacity of the active films

The loading capacity of the three cellulose derived polysaccharides (CMC, HPMC and MC) was studied as a function of PA content in the prepared films versus its initial content in the FFS (Fig. 1).

All of the examined films revealed a similar pattern of PA capacity, which fits a logarithmic function. At a particular point the plateau is reached, where increase of PA concentration in the FFS does not lead to an increase in the final films’ PA content. The saturation concentration of PA in the final films was determined as its maximal loading capacity and calculated as an average of the last four data points of the plateau region. The maximal PA content in different cellulose derivatives used in this research was found to be very similar at 2.92–3.44% w/w. It was found that PA’s content in the films is greatly enhanced in all cellulose types used upon addition of β-CD. Moreover, the clear correlation between an increase of β-CD concentration and an increase of PA content in the final films was observed. The maximal loading capacity of films that contained 2.5% w/w β-CD was found to be 12.45–16.33% w/w. The maximal loading capacity of films that contained 5% w/w β-CD was found to be 29.08–39.17% w/w. This is approximately a ten-fold increase as compared to the capacity of films without β-CD.

PA release from CMC, HPMC and MC-based films was monitored during 30 days at 20 °C (Fig. 2). In accordance with capacity studies, this experiment also showed that the larger the concentration of β-CD used, the more PA was observed in films both in the beginning of the experiment and through the end of it. At a particular point all films reached a terminal PA plateau value. At this plateau region an effective release of PA has ended and the PA concentration in the remained film persists practically unaltered. β-CD resulted in a two-fold prolongation of PA’s effective release time. Notably, in the films that have no β-CD, PA’s effective release has ended after 3–7 days (for HMPC and CMC-based films, respectively), while in the films that contained 5% w/w β-CD, PA’s effective release has ended after 7–14 days (for HMPC and CMC-based films, respectively). By comparing the area under the curves in Fig. 2, it was calculated that films with 5% w/w β-CD have released 7–17 times more PA than their β-CD free analogs. The area under the curves was calculated...
utilizing integration and was found to be 35.4, 15.1, and 13.0 for films with no β-CD (for CMC, HPMC and MC-based films, respectively) and 249.0, 214.4 and 183.1 (for CMC, HPMC and MC-based films, respectively) for films with 5% w/w β-CD.

Thus, β-CD’s presence has led to a notable enhancement of the films’ loading capacity and improvement in their release features. The beneficial effect can be a result of hydrogen bond interactions between β-CD’s exterior OH groups and PA. In addition, a formation of a β-CD:PA inclusion complex can take place [29]. These interactions encourage PA’s affinity to the polymer matrix. β-CD is usually utilized to encapsulate or dissolve lipophilic molecules. Numerous works report usage of β-CD with hydrophobic guest molecules [22,30]. According to our knowledge, β-CD was not previously used for the delivery and controlled release of hydrophilic molecules.

3.2. Physical and mechanical properties of the films

3.2.1. Mechanical properties

Tensile stress (TS), percent elongation at break (PE) and Young’s modulus (YM) have been measured for the prepared films (Table 1). Determination of the mechanical properties of the prepared films is important to estimate their applicative potential for instance as food packaging materials. Mechanical properties of the original films with no PA and no β-CD have been examined and were found

![Graphs showing PA concentration in films](image)

**Fig. 2.** PA concentration left in films during a 30 day period at 20 °C and RH 65%. Values represent means of three replications and 95% t-based confidence intervals. The letters represent comparisons of the PA amounts in the films with different β-CD content at each X-axis point. The values followed by different letter are significantly different according to Tukey–Kramer HSD test at p ≤ 0.05.

### Table 1

<table>
<thead>
<tr>
<th>Polymer</th>
<th>No β-CD</th>
<th>2.5% β-CD</th>
<th>5% β-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No PA</td>
<td>9.7% PA</td>
<td>No PA</td>
</tr>
<tr>
<td>Tensile stress [MPa]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>57.6 ± 0.6,</td>
<td>61.7 ± 0.5,</td>
<td>1.0 ± 0.2,</td>
</tr>
<tr>
<td>HPMC</td>
<td>36.9 ± 1.6,</td>
<td>28.9 ± 4.3,</td>
<td>0.8 ± 0.2,</td>
</tr>
<tr>
<td>MC</td>
<td>88.1 ± 7.2,</td>
<td>57.1 ± 12.8,</td>
<td>2.6 ± 0.7,</td>
</tr>
<tr>
<td>Percent elongation at break [%]</td>
<td>9.0 ± 1.4,</td>
<td>11.7 ± 0.9,</td>
<td>2.5 ± 0.6,</td>
</tr>
<tr>
<td>HPMC</td>
<td>13.8 ± 2.5,</td>
<td>9.3 ± 1.7,</td>
<td>3.6 ± 0.4,</td>
</tr>
<tr>
<td>MC</td>
<td>42.6 ± 4.8,</td>
<td>22.1 ± 2.9,</td>
<td>10.8 ± 1.1,</td>
</tr>
<tr>
<td>Young’s modulus [MPa]</td>
<td>1748.7 ± 197.9,</td>
<td>1836.6 ± 239.3,</td>
<td>72.1 ± 19.7,</td>
</tr>
<tr>
<td>HPMC</td>
<td>777.5 ± 128.1,</td>
<td>954.5 ± 62.9,</td>
<td>74.2 ± 7.0,</td>
</tr>
<tr>
<td>MC</td>
<td>1384.8 ± 197.4,</td>
<td>1287.2 ± 134.4,</td>
<td>101.1 ± 41.7,</td>
</tr>
</tbody>
</table>

Values represent means of three replications and 95% t-based confidence intervals. The letters represent comparisons between the same cellulose type. The values followed by different letter are significantly different according to Tukey–Kramer HSD test at p ≤ 0.05.
Fig. 3. TGA and DTA for CMC-based films. Temperature profile settings 25–550 °C at 10 °C/min under N₂.

to be in correlation with previously published measurements for cellulose derivatives [31,32].

TS is defined as a material’s resistance to deformation. Our results show that PA’s addition had no significant effect on the films’ TS values. Addition of β-CD alone caused a dramatic decrease to the TS values and the films became more brittle. Interestingly, when β-CD was added in combination with PA, its undesired effect on film quality was subdued. For instance, the TS of CMC-based films was reduced from 57.6 ± 0.6 MPa to 1.0 ± 0.2 MPa upon adding β-CD. Notably when β-CD was added in combination with PA, the films’ TS value was increased to 20.5 ± 0.1 MPa.

A film’s PE relies on its extension at the moment of rupture. Addition of PA did not cause significant change in the PE values. Addition of β-CD with no PA was found to decrease the film’s PE making them less elastic. Similar to TS results, when PA was involved and added together with β-CD, the PE values were notably rehabilitated.

YM is described as a measure of stiffness in an elastic specimen. YM values declined drastically due to addition of 2.5% w/w β-CD in all of the films. CMC and MC-based films’ YM values dropped even further when 5% w/w β-CD was added. As is the case with TS and PE, a decline in YM values due to β-CD addition was diminished greatly when PA was added to the films’ composition. PA’s refining effect was so drastic that in some cases it led to a ten-fold difference in values. For instance, in the case of CMC-based films, their value dropped from 1748.7 MPa to 72.1 MPa when β-CD was added alone. On the other hand, when β-CD was combined with PA, the YM of the film rose to 760.1 MPa.

The mechanical properties of the films were significantly affected by the incorporation of β-CD. Possibly, β-CD interrupts the polymer’s three dimensional packing by a formation of structural assemblies between β-CD’s hydrophobic and hydrogen bonding sites and the cellulose derivatives’ side chains. Such assemblies
were described by Stoddart [33] and they resemble threaded beads in a necklace [31]. The assemblies lead to a less compact three dimensional arrangement and ultimately to thicker films with poorer mechanical properties. As PA takes up β-CD’s binding sites, β-CD molecules are occupied by interactions with PA and form less assemblies with polymer side chains and the film matrix architecture remains less affected.

This observation points to a synergetic effect between PA and β-CD. β-CD enabled the greater upload capacity and a more effective release of PA from the cellulose-derived films. PA in turn subdued the negative effect caused by β-CD and allowed formation of films with considerable mechanical properties. According to our knowledge, this is the first demonstration of a synergetic effect between β-CD and its guest molecule where not only β-CD but also the guest molecule benefits the system.

WVTR of the films was studied (Table 2).

WVTR is a crucial physical parameter and responsible for film humidity resistance. Addition of active components can affect the polymer film’s WVTR and such effects are not always desirable. In the present case, addition of PA did not affect the prepared films’ WVTR. β-CD’s addition to the films’ composition did not cause drastic changes in WVTR values for the most part. HPMC-based films were an exception and showed a significant decrease of WVTR values upon adding β-CD to their composition, demonstrating an enhanced humidity resistance. Testing WVTR for films with high content of β-CD and without PA was not possible due to the films’ brittle state.

### 3.2.2. TGA and DTA analysis

PMC-based films were studied for their thermal profile using TGA and DTA analyses (Fig. 3). Analysis of the films’ DTA graphs showed that release of the water content when β-CD was present was delayed to higher temperatures. This suggests that β-CD holds water by creating hydrogen bonds with its hydroxyl groups [26]. As the temperature increased, the thermograms revealed the decomposition profile of the films. Addition of PA did not alter the films’ decomposition profile significantly as compared to original polysaccharides’ profiles. Films that contained β-CD have shown a drastically different thermal decomposition profile. It is notable that when β-CD was added in combination with PA, the films’ thermal profile tended to return and become more similar to the original films. This behavior is another indication of the synergetic effect that was observed between β-CD and PA; an inhibition of PA release by β-CD and at the same time a minimized physical change of the films due to PA’s presence along with β-CD.

### 3.2.3. Film structure and morphology

The presence of PA was also found to diminish the undesired effects of β-CD on the films’ morphology. PA alone did not lead to
Fig. 5. ESEM images of: (a) CMC-based film (MAGX1000); (b) CMC-based film with 9.7% w/w PA (MAGX1000); (c) CMC-based film with 2.5% w/w β-CD (MAGX250); (d) CMC-based film with 2.5% w/w β-CD and 9.7% w/w PA (MAGX1000).

Fig. 6. Antimicrobial activity. Fungal contamination development on wheat grains on potato dextrose agar (PDA) plates after 30 days of storage under various treatments.
notable changes in the films’ morphology. Addition of β-CD has led to spherulitic crystallization that was observed in all of the films regardless of cellulose type. This crystallization appeared to occur above the cellulose platform. Similar crystallizations were reported before in chitosan-based films [33]. Addition of β-CD in combination with PA has led to a positive effect on the films’ morphology in accordance with previous observations concerning the films’ mechanical properties. Films with PA and β-CD appeared to be much smoother and homogenous under the light microscope (Fig. 4). ESEM images of CMC-based films confirm β-CD crystallization above the polymer matrix. It appears that addition of PA assists in minimizing the films’ texture change (Fig. 5). This is another indication of the synergistic effect between PA and β-CD.

3.3. Antimicrobial activity

Two types of CMC-based films were tested for their antimicrobial activity on post-harvest fresh grains across a time period of 30 days (Fig. 6). Films that contained PA were found to inhibit fungal growths. It can be seen that films that contained PA and β-CD resulted in a more effective and prolonged inhibition as compared to films that contained PA with no β-CD (100% fungal infection after 15 days rather than 7 days, respectively), but less effective than the pure PA treatment. These results are crucial for implementing controlled release systems. The presented antimicrobial films may provide a viable basis for effective future technology. Pure PA is currently dispersed on grains during their post-harvest storage [34]. Repetitive dispersions are needed because of PA’s volatile nature. The presented encapsulation systems have shown the ability to inhibit PA’s release rate, allowing the cancelation of the repetitive treatments in the future. Furthermore, in the presented formulations PA appears as solid films and not as a liquid, allowing its safer utilization.

4. Conclusions

A series of active films based on cellulose derived hydrocolloids (CMC, HPMC and MC) was prepared. PA was incorporated into the film matrix as a volatile antimicrobial agent. The films’ loading capacity was found to be greatly amplified upon addition of β-CD. Moreover, β-CD also prolonged the duration of PA release. β-CD negatively affected the mechanical, physical and morphological properties of the films. Notably, β-CD’s negative effects were found to be significantly subdued upon the presence of PA. Films containing β-CD and PA inhibited fungal growth on grains and therefore may be considered as an alternative treatment for liquid PA.

Thus, in this work we have shown for the first time that β-CD can be effectively utilized for the successful delivery and controlled release of hydrophilic molecules in hydrocolloids-based matrices. PA diminishes β-CD’s negative effects on mechanical, physical and morphological properties and allows the formation of good quality films. This is an original demonstration of a synergetic effect between β-CD and its guest molecule where both compounds benefit the active film. We hope our findings will encourage the research of hydrocolloids-based antimicrobial films for a formation of safe biodegradable controlled release systems and will contribute for their utilization in the field of active packaging.

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