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Antimicrobial citric acid/poly(vinyl alcohol) crosslinked films: Effect of cyclodextrin and sodium benzoate on the antimicrobial activity

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ABSTRACT

In this paper, poly(vinyl alcohol) (PVOH) cast films were crosslinked with citric acid (CTR) and hydroxypropyl-β-cyclodextrin (HPβCD) and loaded with sodium benzoate (NaBz) as preservative agent, with different compositions of CTR (10–30 wt%) and NaBz (0–6 wt%) with or without HPβCD (10%). Permeability tests showed that the presence of CTR and HPβCD does not modify PVOH barrier properties. Before incorporation, the complex formation of HPβCD with NaBz was investigated through 2 dimensional Nuclear Magnetic Resonance, which revealed a 1:1 stoichiometry. Raman spectroscopy cartography showed that the NaBz distribution in the films was homogeneous, especially with HPβCD. The NaBz release in water is prolonged with HPβCD on one hand for high crosslinking times (360 min) and, on the second hand, in the assays carried out at low temperature (4 °C). The released quantity is increased from 40% to 70% when NaBz is included into HPβCD cavity. Antimicrobial assays were performed against *Staphylococcus aureus*, *Escherichia coli* and *Candida*. Without HPβCD, all films present a contact antimicrobial activity thanks to grafted CTR and the best diffusion activity was obtained for 6 wt% NaBz for the three microorganisms. With HPβCD, the antimicrobial activity by diffusion was increased with crosslinking times.

1. Introduction

In recent years, the elaboration of active packaging from biodegradable polymers has known a growing interest, especially due to their environmentally friendly properties. Among them, poly(vinyl alcohol) (PVOH) exhibits good physical properties and in particular high barrier properties to oxygen and carbon dioxide (Finch, 1992). However, its permeability to gases may be dramatically increased due to its tendency to absorb humidity, which can act as a plasticizer of the framework. Considering the high-moisture conditions applied in many packaging cases, it is then necessary to crosslink the PVOH matrix to ensure sufficient mechanical handling. To that purpose, the effectiveness of chemical crosslinking agent such as citric acid (CTR), accepted as food additive, has been proven (Birck, Degoutin, Tabary, Miri, & Bacquet, 2014; Musetti et al., 2014; Wang, Ren, Li, Sun, & Liu, 2014).

Active packaging systems are now developed with additional properties, including antioxidant and antimicrobial properties (Appendini & Hotchkiss, 2002; Gómez-Estaca, López-de-Dicastillo, Hernández-Muñoz, Catalá, & Gavara, 2014), aiming at extending shelf-life and food safety by controlling the growth of pathogenic microorganisms. In the case of antimicrobial films, the polymer matrix may be loaded with antimicrobial compounds (Sung et al., 2013), such as essential oils (Higueras, López-Carballo, Gavara, & Hernández-Muñoz, 2015; Kurek, Moundanga, Favier, Galić, & Debeaufort, 2013; Musetti et al., 2014), peptides (Imran, Klouj, Revol-Junelles, & Desobry, 2014; Neetoo & Mahomoodally, 2014) or preservatives (Buonocore, Del Nobile, Panizza, Corbo, & Nicolais,

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2003; Mondal et al., 2015; Neetoo & Mahomoodally, 2014; Sun, Wang, Kadouh, & Zhou, 2014). Among the latter, sodium benzoate (NaBz) is the most effective preservative against microorganisms, including *Staphylococcus aureus* and *Escherichia coli* (Chipley, 1993). The efficiency of antimicrobial activity depends on the release rate and kinetics, which may be improved by introducing in the polymer matrix some specific carriers such as cyclodextrins, interacting with the antimicrobial compounds as reservoirs for the extended release (Higueras et al., 2015; López-de-Dicastillo, Gallur, Catalá, Gavara, & Hernandez-Muñoz, 2010; Raouche, Mauricio-Iglesias, Peyron, Guillard, & Gontard, 2011; Rutenberg, Bernstein, Paster, Fallik, & Poverenov, 2015; Sun, Sui, et al., 2014; Vega-Lugo & Lim, 2009). In these previous studies, cyclodextrins are not covalently bonded to the polymer matrix.

Cyclodextrins (CDs) are biodegradable cyclic oligosaccharides consisting of a three-dimensional structure with a hydrophobic inner cavity and a hydrophilic outer surface, which allows the formation of stable inclusion complexes with many organic compounds. On one hand, the solubilization of the active compound in the initial solution is then improved, which results in an enhanced loading capacity of the film. On the other hand, the stability of the inclusion complex leads to a prolonged delivery of the entrapped active agent. Moreover, we reported an innovative approach by co-crosslinking PVOH and CDs by CTR to form insoluble films (Birck et al., 2014). In particular, we extensively studied the crosslinking reaction and showed that the presence of hydroxypropyl-β-cyclodextrin (HPβCD), a water soluble cyclodextrin derivative, had no major effect on the physico-chemical and mechanical properties of PVOH/CTR films.

The aim of this work was to study antimicrobial PVOH/CTR films containing HPβCD for the release of sodium benzoate and a surface activity through free COOH groups from CTR, as depicted on Fig. 1. The preliminary formation of HPβCD/NaBz complex was characterized by nuclear magnetic resonance (NMR) and the presence and distribution of NaBz in the films were investigated through Raman spectroscopy. The release of NaBz in water was studied as a function of the crosslinking parameters, such as the proportion of CTR and the crosslinking time. The effect of HPβCD and the loading capacity on the release was also investigated. Finally, the antimicrobial activity of the films was tested *in vitro* against two food pathogen bacteria, *S. aureus* and *E. coli*, as well as against a yeast, *Candida albicans*.

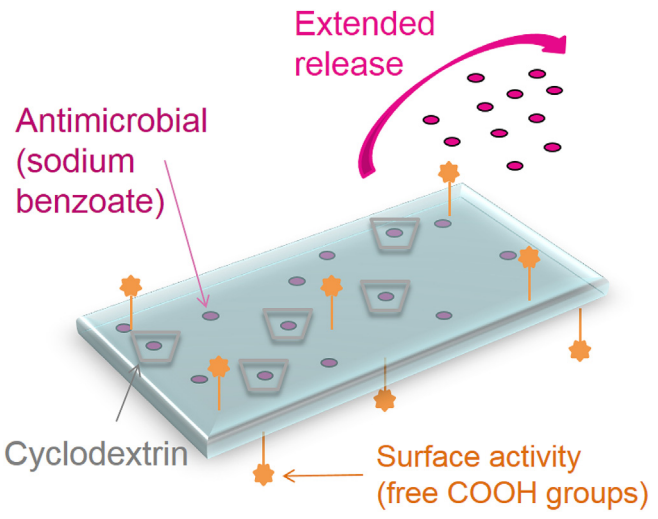

Fig. 1. Schematic representation of antimicrobial PVOH/HPβCD/CTR films with surface activity and extended release of NaBz.

2. Materials and methods

2.1. Materials

Poly (vinyl alcohol) (PVOH, M_w = 31,000–50,000 g mol^{-1}, 87–89% hydrolysis, powder) and citric acid (CTR, food additive European code E330) were purchased from Aldrich Chemicals (St. Quentin Fallavier, France). Hydroxypropyl-β-cyclodextrin (HPβCD, Kleptose®, MS = 0.65, highly soluble in water) was provided by Roquette (Lestrem, France) as well as maltodextrin (MX, Glucidex®). The mean Molar Substitution (MS) is the average number of hydroxypropyl groups per anhydroglucose unit. Sodium benzoate (NaBz, food additive European code E211) was purchased from Prolabo (Fontenay-sous-Bois, France).

2.2. Elaboration of PVOH crosslinked films without preservative

All films were prepared by solvent casting method as described in a previous paper at 130 ± 1 °C during 40 or 360 min (Birck et al., 2014). The relative weight proportion of CTR in PVOH solution was set to 10, 20 or 30 wt%.

Crosslinked PVOH/HPβCD/CTR films were prepared following the same protocol as for films without HPβCD. The proportion of HPβCD in the film was fixed considering first an equimolar complex between HPβCD and the added antimicrobial agent and secondly according to the European directive giving a limit for constituent's migration in food packaging at 10 mg/dm^2 (85/572/EEC, Council Directive of 19 December 1985). Thus, films containing 10 wt% of HPβCD and 20 wt% CTR were prepared.

The thickness of each film was 120 ± 20 μm, except for antimicrobial study where the thickness was 500 ± 150 μm, in order to ensure no layover of the film after gelosis deposition in the modified Kirby Bauer test.

2.3. Elaboration of PVOH crosslinked films loaded with sodium benzoate

The same protocol as described above was followed. For films without HPβCD, NaBz was added in the initial mixture containing PVOH and CTR (20 wt%) at different weight proportions (1, 3 or 6 wt %). For films with MX (10 wt%), an equimolar mixture [MX-NaBz] previously prepared is added. For films with HPβCD (10 wt%), NaBz was introduced in the mixture complexed with HPβCD.

The complex [HPβCD:NaBz] 1:1 was obtained from a solution containing HPβCD and NaBz in distilled water at 0.158 mol/L concentrations that was stirred overnight at room temperature and freeze-dried.

As previously, the thickness of each film was 120 ± 20 μm, except for antimicrobial study where the thickness was 500 ± 150 μm.

2.4. Nuclear magnetic resonance (NMR)

The inclusion complex between HPβCD and NaBz was characterized by ^{1}H NMR. All experiments were recorded using a spectrometer Bruker Avance™ 400 (Bruker Corporation, Germany) equipped with a Bruker Ultra-Shield 9.4 T (proton Larmor frequency of 400.33 MHz). We used a BBI probe (1H, X). Two-dimensional ROESY experiments (Hwang & Shaka, 1992), used to determine the depth penetration of the guest molecule into the HPβCD cavity, were acquired in the phase sensitive mode using the Bruker ASX 400 (9.4 T) spectrometer. The probe temperature was regulated to 300 K. Each spectrum consisted of a matrix of 2 K (F2) by 2 K (F1) covering a sweep width of 4084 Hz. We used spin-lock mixing periods of 500 ms. The stoichiometry of the complex was