Carboxymethylcellulose acetate butyrate/poly(4-vinyl-*N*-pentyl pyridinium bromide) blends as antimicrobial coatings

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Abstract. Blends of carboxymethyl cellulose acetate butyrate (CMCAB), a cellulose derivative, and poly(4-vinyl-*N*-pentyl pyridinium bromide) (QPVP-C5), an antimicrobial polymer, were prepared by casting method and characterized by means of Fourier transform infrared vibrational spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and contact angle measurements. Miscibility between CMCAB and QPVP-C5 was evidenced by DSC measurements of blends, which showed a single thermal event of T_g , and SEM images, which revealed homogenous morphology, regardless the blend composition. Moreover, thermal stability of QPVP-C5 was substantially enhanced, when it was mixed with CMCAB. Upon increasing the QPVP-C5 content in the blend the wettability and antimicrobial activity against Gram-positive bacteria *Micrococcus luteus* increased, indicating the surface enrichment by pyridinium groups. In fact, blends with 70 wt% QPVP-C5 reduced 5 log and 4 log the colony-forming units of *Micrococcus luteus* and *Escherichia coli*, respectively.

Keywords: coatings, carboxymethylcellulose acetate butyrate, poly(4-vinyl-*N*-pentyl pyridinium bromide), antimicrobial properties, blends

1. Introduction

Carboxymethylcellulose acetate butyrate (CMCAB) is a cellulose ester often applied as coating for cellulosic substrates, matrix component of compression tablets or support for biomolecules [1–5]. Although CMCAB has been successfully used as coating material, it could be improved to gain antimicrobial property. Such property might be achieved by immobilizing lysozyme, an enzyme that catalyzes the hydrolysis of peptidoglycans on bacterial cell walls, on the surface of CMCAB, so that the catalytic activity is high enough to kill bacteria, even after many cycles of use [5]. The incorporation of antimicrobial agents,

like silver ions or silver nanoparticles [6, 7], antibiotics or quaternary ammonium compounds (QAC) [8], to polymeric matrices is also a common strategy to obtain polymeric surfaces with microbiocidal activity. Particularly the polymers containing QAC in the backbone or sidechains, such as chitosan [9, 10], *N*-alkyl polyethyleneimines [11, 12] and poly(4-vinyl-*N*-alkylpyridinium bromide) with alkyl chains shorter than six carbons [13–18], have been applied as bactericidal materials. The mechanism of their biocide action is well reported in the literature [14, 19–21]. The adsorption of bacteria onto cationic surfaces is driven by electrostatic interac-

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tion between polymer cationic charges and the bacterial membranes negative charges (teichoic acids of Gram-positive bacteria and negatively charged phospholipids at the outer membrane of Gram-negative bacteria). Upon adsorbing, the compensation of the negative charges of the bacterial envelope is provided by the cationic charges of the substrate, and the bacteria lose their natural counter ions, which are released, increasing the entropic gain. Thus, solid substrate becomes biocidal when the number of cationic sites is large enough to remove counter ions from bacteria, inducing disruption of the bacteria envelope. Moreover, if the polycation chains have hydrophobic side chains, the disruption of outer and cytoplasmic membranes is favored.

Considering the demand for long-lasting antimicrobial coatings based on environmentally friendly and non-toxic products [22], blends of CMCAB and poly(4-vinyl-*N*-pentylpyridinium bromide), QPVP-C5, were prepared by casting in a broad composition range. The morphological structure, miscibility, thermal behavior and wettability were investigated by means of scanning electron microscopy (SEM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and contact angle measurements, respectively. Antimicrobial properties of neat CMCAB and CMCAB/QPVP-C5 blends against Gram-positive bacteria *Micrococcus luteus* were assessed by changes of microbial dispersions turbidity. Moreover, the ability to reduce the colony-forming units (CFU) of *Micrococcus luteus* and *Escherichia coli* was quantitatively evaluated for neat CMCAB and CMCAB/QPVP-C5 blends.

2. Experimental section

2.1. Materials

Carboxymethyl cellulose acetate butyrate (CMCAB-641-0.2) ($\bar{M}_n = 20\,000\text{ g}\cdot\text{mol}^{-1}$; $DS_{Ac} = 0.4$; $DS_{Bu} = 1.6$ and $DS_{CM} = 0.3$), was kindly supplied by Eastman Chemical Co. (Brazil). The chemical structure of

Figure 1. Representation of chemical structures of CMCAB, where R refers to H, COCH₃, COC₃H₇ and/or CH₂COOH

CMCAB is represented in Figure 1. Poly(4-vinylpyridine) (PVP, $\bar{M}_w \sim 60\,000\text{ g}\cdot\text{mol}^{-1}$, degree of polymerization, DP , ~ 600) and 1-bromopentane (purity 99%) were obtained from Sigma-Aldrich (Brazil), and anhydrous ethanol (98%), diethyl ether (99%) and HBr (48%) from Labsynth (Brazil). All reagents and solvents were used without previous purification. Gram-positive bacteria *Micrococcus luteus* (ATCC 4698) and Gram-negative bacteria *Escherichia coli* (ATCC25922) were purchased from Adolf Lutz Institute (Brazil).

2.2. Preparation of poly(4-vinyl-*N*-pentylpyridinium bromide), QPVP-C5

Bromide salt of PVP quaternized with linear pentyl chains was prepared in a flask with a reflux condenser by dissolving PVP (10 wt%) in anhydrous ethanol, adding the 1-bromopentane with an excess of 5 times the stoichiometric amount and keeping the mixture stirring under nitrogen atmosphere for 24 h at 60°C [23, 24], as schematically represented in Figure 2. The mixture was then precipitated in diethyl-ether to obtain a slightly yellow solid that was re-dissolved in ethanol and re-precipitated the same way. The product was washed with a cold HBr 0.1 mol·L⁻¹ solution in order to remove any eventual unreacted parts, washed with diethyl-ether once more, and dried under vacuum at room temperature. The effectiveness of the quaternization was evaluated by Fourier transform infrared (FTIR) vibrational spectroscopy observing the shift of the characteristic pyri-

Figure 2. Schematic representation of quaternization of PVP with bromopentane

dine N–C stretching band from 1600 to 1640 cm^{-1} , which is typical for pyridinium cations. The band in 1600 cm^{-1} disappeared entirely, indicating complete quaternization. The resulting polycation was coded as QPVP-C5.

2.3. Preparation of CMCAB/QPVP-C5 blend films

CMCAB/QPVP-C5 blends were prepared by dissolving the polymers in ethanol (1 wt %), which is a good solvent for both polymers and casting the solutions (10 mL) in polyethylene (PE) dishes (4 cm diameter) at 50°C, during 24 h. PE dishes were chosen because when glass Petri dishes were used for polymer film formation, the adhesion to glass surface was so strong that impeached the films removal after drying. The weight fractions (x) of QPVP-C5 in the blends were 0; 0.17; 0.30; 0.50; 0.70 and 1.0.

2.4. Characterization

The CMCAB/QPVP-C5 blends films were analyzed by Fourier transform infrared vibrational spectroscopy (FTIR) in Bomem MB100 equipment, resolution of 4 cm^{-1} . The morphology of CMCAB/QPVP-C5 blends was analyzed by means of scanning electron microscopy with FE-SEM JEOL 7401 equipment. Cryofractured samples were coated with a thin (2 nm) gold layer prior to SEM analyses. Thermogravimetric analyses (TGA) of pure polymers and CMCAB/QPVP-C5 blends were carried out in TGA 2950 equipment (TA Instruments). Pt crucibles containing the samples were heated and cooled down at rate of 10°C·min⁻¹, ranging from 25 up to 500°C, under N₂ atmosphere (50 mL·min⁻¹). Glass transition temperature (T_g) of pure polymers and respective blends were determined by means of differential scanning calorimetry (DSC) in TA-DSC Q10V9.0 equipment. Each sample sealed in Al crucibles was heated and cooled down at rates of 15 and 20°C·min⁻¹, respectively, in the temperature range from 25 to 250°C under dynamic N₂ atmosphere (50 mL·min⁻¹). The DSC cell was calibrated with In ($T_m = 157^\circ\text{C}$; $\Delta H_m = 28.54 \text{ J}\cdot\text{g}^{-1}$) and Zn ($T_m = 420^\circ\text{C}$). An empty crucible was used as reference. The second heating was considered for the determination of T_g values. Contact angle measurements were performed at (25±1)°C in a home-built apparatus [25]. Sessile drops of 8 μL Milli-Q water were used for the advancing contact angle (θ_a) measurements for pure polymers, QPVP-C5 and CMCAB,

and CMCAB/QPVP-C5 blends films. Three samples of the same composition were analyzed at two different spots.

2.5. Determination of biocidal action

2.5.1. Monitoring the turbidity of *Micrococcus luteus* dispersions

The antimicrobial action of pure CMCAB, pure QPVP-C5, and CMCAB/QPVP-C5 blends was assessed by a standard assay for the determination of lysozyme activity, as described elsewhere [26]. Briefly, the initial turbidity (τ_i) of aqueous dispersions (pH 6) of *Micrococcus luteus* (ATCC 4698) at 0.5 $\text{mg}\cdot\text{mL}^{-1}$ was determined at 25°C and 650 nm, using Beckmann Coulter DU-600 spectrophotometer. Polymer films (22 cm^2) were dipped into the *M. luteus* dispersions and allowed to interact during 1 h. After this period of time the turbidity (τ_f) of bacteria dispersions was measured again. Since *M. luteus* is micrometer-sized and the disruption of bacteria envelope leads to decrease in size, antimicrobial activity can be correlated with the relative decrease of turbidity ($\Delta\tau$); the larger is $\Delta\tau$, the more efficient is the antimicrobial agent (Equation (1)):

$$\Delta\tau = \frac{\tau_i - \tau_f}{\tau_i} \cdot 100 \quad (1)$$

As a control experiment, ($\Delta\tau$) values were also measured after 1 h for aqueous dispersions of *M. luteus* (τ_i) in the absence of any polymer.

2.5.2. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of QPVP-C5 for *Micrococcus luteus* and *Escherichia coli*

The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of QPVP-C5 for *E. coli* (ATCC 25922) and *M. luteus* (ATCC 4698) were determined in triplicates by the microdilution broth method in 96-well microplates, as recommended by the CLSI [27]. In brief, twofold serial dilutions of QPVP-C5 were prepared at final volume of 0.1 mL of Mueller-Hinton broth per well in a 96-well microtitre plates. The final concentration of the QPVP-C5 ranged from 6000 to 0.005 $\mu\text{g}\cdot\text{mL}^{-1}$. Each well of the microtitre plate was inoculated with bacterial cell suspension to a final concentration of $5\cdot 10^5 \text{ CFU}\cdot\text{mL}^{-1}$ and the plates were incubated at 35°C for 16 to 20 hours in an ambient air

incubator. MIC was defined as the lowest concentration of QPVP-C5 that completely inhibited growth of the organism (lack of turbidity) in the microdilution wells. To determine the MBC, 10 μL aliquots were taken from selected wells that did not show visible bacterial growth and spread on plates containing Agar Muller-Hinton medium, being incubated for 24 hours at 37°C. Visual observation indicated presence or absence of growth and MBC endpoint was considered as the lowest concentration of QPVP-C5 solution showing no growth.

2.5.3. Colony forming units (CFU) count on CMCAB and CMCAB/QPVP-C5 blends

The antibacterial activity of CMCAB and CMCAB/QPVP-C5 blends, against *M. luteus* ATCC 4698 and *E. coli* ATCC 25922 was essentially determined as described in the JIS Z 2801:2000 standard [28]. An aliquot (400 μL) of a cell suspension of either *M. luteus* ATCC 4698 ($10^5 \text{ CFU} \cdot \text{mL}^{-1}$) or *E. coli* ATCC 25922 ($10^6 \text{ CFU} \cdot \text{mL}^{-1}$) was held in intimate contact with each of the 2 replicates of the test surfaces, i.e., CMCAB or CMCAB/QPVP ($x = 0.7$) films 22 mm of diameter, prepared using sterile 12-well flat bottom plates, for 24 hours at 37°C, under humid conditions. Next, the size of surviving bacterial populations was determined by viable cell counts on Trypticase Soy Agar, after incubation at 37°C for 24 hours of 0.1 mL sample taken from the test surfaces. A negative control experiment (without any bacteria) was also performed.

2.6. Quantification of QPVP-C5 leaching

The amount of QPVP-C5 leached during antimicrobial assays was evaluated by UV-Vis analyses following the same conditions used with bacteria dispersions. Polymeric blend films were dipped in distilled water and after 1 h the concentration of polycation was quantified in water by measuring the absorption intensity at $\lambda = 258 \text{ nm}$ using Beckmann Coulter DU-600 spectrophotometer. The amount of QPVP-C5 leached was determined through an analytical curve obtained from five different concentrations of specimen at the wavelength 258 nm, which is assigned to electronic transitions in the pyridinium ring due to the $\pi \rightarrow \pi^*$ electronic transition.

3. Results and discussion

3.1. CMCAB/QPVP-C5 blends characterization

FTIR spectra obtained for all CMCAB/QPVP-C5 blends presented the typical absorption bands observed for the corresponding pure CMCAB and QPVP-C5 polymers (Figure 3), namely, the C=O stretch of cellulose ester at 1750 cm^{-1} and the pyridinium group at 1640 cm^{-1} . Considering that strong acid-base interactions can cause shifts of up to 30 cm^{-1} in the carbonyl and carboxylic acid bands [29], the spectra in Figure 3 indicate the absence of such interactions. Liu and Xiao characterized blends of konjac glucomannan gum [30] or chitosan [31] with QPVP-C4 by means of FTIR and other analytical techniques, which evidenced intermolecular interactions between the polymers in the both blends. Whereas in the first case the stretching vibration of –OH in comparison to the pure konjac glucomannan gum shifted gradually to higher wavenumbers as the amount of QPVP-C4 increased, in the latter the –NH₂ band of chitosan split in two denoting interaction with QPVP-C4.

The miscibility between CMCAB and QPVP-C5 was investigated by DSC analyses. The glass transition temperature (T_g) values determined for pure CMCAB, pure QPVP-C5 and blends are presented in the Table 1. DSC curves (Figure 4a) exhibited a sin-

Figure 3. FTIR spectra of pure QPVP-C5 and CMCAB, and CMCAB/QPVP-C5 blends. ‘x’ stands for the weight fraction of QPVP-C5 in the blend. The dashed lines indicate the typical absorbance bands of pyridinium group and C=O stretch of ester groups at 1640 and 1750 cm^{-1} , respectively.

Table 1. Experimental glass transition temperature (T_g), glass transition temperature calculated with Fox equation (T_g^F), contact angle (θ) values and turbidity decrease ($\Delta\tau$) determined for pure CMCAB, pure QPVP-C5 and their blends. 'x' stands for weight fraction of QPVP-C5 in the blends

Sample	x	T_g [K]	T_g^F [K]	θ [°]	$\Delta\tau$ [%]
QPVP-C5	–	350.6±0.5	–	*	96±2
CMCAB/QPVP-C5	0.7	351±1	364	**	66±5
CMCAB/QPVP-C5	0.5	370±1	380	**	52±2
CMCAB/QPVP-C5	0.3	370±1	389	65±3	30±2
CMCAB/QPVP-C5	0.17	–	–	69±4	10±1
CMCAB	–	419±1	–	71±2	3±1

Films were either (*) dissolved or (**) swollen by droplets of water.

gle T_g value, indicating miscibility of systems. The T_g value of miscible polymer blends can be estimated by the Fox equation (Equation (2)) [32]:

$$\frac{1}{T_g} = \frac{x_1}{T_{g1}} + \frac{x_2}{T_{g2}} \quad (2)$$

where x_1 and x_2 are the weight fraction of each polymer in the blend and T_{g1} and T_{g2} are the T_g s of polymer *a* and polymer *b*, respectively.

For all blends the experimental value of T_g was smaller than the predicted one, as presented in Figure 4b. The negative deviation from Fox equation indicates good miscibility between the two components [32]. Hydrophobic interactions between CMCAB butyl groups ($DS_{Bu} = 1.64$) and QPVP-C5 pentyl groups might possibly drive the miscibility between the polymers, since no shift in the C=O or pyridinium absorption bands could be observed in

the FTIR spectra of blends in comparison to the spectra of pure polymers.

SEM images obtained for cryofractured films of pure CMCAB and QPVP-C5 (Figure 5a and 5b, respectively), and CMCAB/QPVP-C5 blends with $x = 0.3$; 0.5 and 0.7 (Figure 5c, 5d and 5e, respectively), showed homogeneous phases in all blends composition, corroborating with the DSC results. The images in the insets allow estimating the film thickness, which ranged from ~15 to ~50 μm .

The thermal stability of pure CMCAB and QPVP-C5 was investigated by means of thermogravimetric (TG) curves, as shown in Figure 6a. The events related to the polymers thermal behavior can be better identified in the derivatives weight loss (DTG) curves (Figure 6b). The TG and DTG curves obtained for CMCAB showed a weight loss stage with peak temperature at 355°C, which can be attributed to the thermal degradation of cellulose ester and corresponded to ~80% of initial weight. Between 420 and 500°C the weight remained constant, since the residual material (~15% of initial weight) was mainly ashes. The TG and DTG curves obtained for QPVP-C5 indicated three stages. The first one corresponding to ~10% weight loss at 59°C was attributed to the loss of ethanol molecules entrapped in the bulk material. The second and third stages with peak temperatures at 290 and 336°C, respectively, were attributed to the decomposition of QPVP-C5, remaining ~5% of initial weight as ashes.

The TG and DTG curves obtained for CMCAB/QPVP-C5 blends are presented in Figures 6c and 6d, respectively. The general trend is that the ther-

Figure 4. (a) DSC curves obtained for pure QPVP-C5, pure CMCAB, and CMCAB/QPVP-C5 blends. (b) Glass transition temperature (T_g) of pure QPVP-C5, pure CMCAB, and CMCAB/QPVP-C5 blends determined by DSC experiments (squares) and calculated (---) by Fox equation as function of weight fraction of QPVP-C5 in the blends (x).

Figure 5. SEM images obtained for pure CMCAB (a), pure QPVP-C5 (b), CMCAB/QPVP-C5 blends with $x = 0.3$ (c), $x = 0.5$ (d) and $x = 0.7$ (e). The scales bars correspond to 2 μm . The scale bars in the insets correspond to 10 μm .

mal stability of the blends was inferior to that of pure CMCAB, but superior to that of pure QPVP-C5. It implies that the thermal stability of QPVP-C5 is enhanced due to the introduction of the CMCAB. A similar behavior was observed for blends of QPVP-C4/ konjac glucomannan, where the polysaccharide enhanced the blends thermostability [30].

3.2. Antimicrobial activity of CMCAB/QPVP-C5 blends

In general polycations are biocidal polymers, but the structural characteristics such as molecular weight, type and degree of alkylation, and distribution of charge affect the antimicrobial action [33]. For instance, the antimicrobial activity of poly(4-vinyl-

Figure 6. TG (a) and DTG (b) curves determined for pure CMCAB (dash line) and QPVP-C5 (solid line). TG (c) and DTG (d) curves obtained for CMCAB/QPVP-C5 blends with $x = 0.3$ (dash line), 0.5 (solid line) and 0.7 (dash-dot line).

N-alkyl pyridinium) bromide decreases for alkyl side group larger than C6 [13]. The reason for this is a tilt in the geometry; when the alkyl chain is 6 carbons long or shorter, the dihedral angle is 0 or 180°, *i.e.* all the atoms lie in the same plane [24]. From the seventh atom forward the alkyl chain becomes crooked making a dihedral angle different from 0 or 180° [24], shielding the positive charge.

The MIC and MBC of QPVP-C5 for both *M. luteus* and *E. coli* amounted to 11.7 $\mu\text{g}\cdot\text{mL}^{-1}$. Such low concentration values indicated that QPVP-C5 has outstanding biocidal properties.

The bactericidal activity of pure CMCAB, pure QPVP-C5 and CMCAB/QPVP-C5 blends was correlated to the relative decrease of turbidity ($\Delta\tau$) of *M. luteus* aqueous dispersion. Table 1 shows the $\Delta\tau$ values measured after interacting during 1 h with each polymeric film. In the case of pure QPVP-C5, chains dissolved in aqueous dispersion, presenting maximal $\Delta\tau$ values ($96\pm 2\%$), which is in agreement with studies previously reported [16]. The $\Delta\tau$ values measured for the CMCAB/QPVP-C5 blends

decreased with the decrease of QPVP-C5 content in the blends. The best antimicrobial effect was observed for the films of CMCAB/QPVP-C5 $x = 0.7$, with $\Delta\tau = 66\pm 5\%$. In other words, the lower was the polycation content in the blends, the less bactericidal was the film. Additionally, the UV-Vis analysis indicated that the amount of leached QPVP-C5 was less than 1.5% of original amount used in the blend preparation, which corresponded to less than 15 $\mu\text{g}\cdot\text{mL}^{-1}$. Considering that it is very close to the MIC and MBC found for QPVP-C5 against *M. luteus* and *E. coli* (11.7 $\mu\text{g}\cdot\text{mL}^{-1}$), the biocidal properties of CMCAB and CMCAB/QPVP-C5 ($x = 0.7$) films were determined directly on the polymer. The CFU reduction was quantitatively determined for *M. luteus* and *E. coli* after 24 h, as shown in Table 2. Against *M. luteus* neat CMCAB presented a modest 2 log reduction, while the blend presented a remarkable 5 log reduction. Against *E. coli* neat CMCAB had no biocidal activity, the CFU increased 1 log; however, the CMCAB/QPVP-C5 ($x = 0.7$) films caused 4 log CFU reduction, evidencing the out-

Table 2. CFU reduction determined for *M. luteus* and *E. coli* after 24 h contact with CMCAB and CMCAB/QPVP-C5 ($x = 0.7$) films

	<i>Micrococcus luteus</i> ATCC 4698 [CFU/mL]			
	0 h	24 h	log CFU reduction	% reduction
CMCAB	$1 \cdot 10^5$	$5.75 \cdot 10^3$	2	94.25
CMC/QPVP-C5 ($x = 0.7$)	$1 \cdot 10^5$	0	5	>99.9
Control	0	0		
	<i>Escherichia coli</i> ATCC 25922 [CFU/mL]			
	0 h	24 h	log CFU reduction	% reduction
CMCAB	$1.0 \cdot 10^6$	$6.7 \cdot 10^7$	–	–
CMC/QPVP-C5 ($x = 0.7$)	$1.0 \cdot 10^6$	$7 \cdot 10^2$	4	>99.9
Control	0	0		

standing biocidal properties for Gram-positive and Gram-negative bacteria.

In order to gain insight about the orientation of pyridinium groups on the CMCAB/QPVP-C5 films, contact angle values (θ) were measured for pure CMCAB and blends with $x = 0.17$ and 0.30 of QPVP-C5, as presented in Table 1. The θ values decreased from $(71 \pm 2)^\circ$ for pure CMCAB to $(65 \pm 3)^\circ$ for blends with $x = 0.30$, indicating a slight increase in the wettability with increasing QPVP-C5 content in the blend. It was not possible to measure the contact angles for blends with $x \geq 0.5$ because the films became swollen by water droplets, invalidating the method. This behavior corroborates with the trend observed for the biocidal activities and supports the idea that upon increasing the QPVP-C5 content in the blends, the pyridinium groups tend to enrich the film surface.

4. Conclusions

Miscible blends of CMCAB/QPVP-C5 were prepared in ethanol, a good solvent for both polymers. The thermal stability of CMCAB/QPVP-C5 blends was superior to that of pure QPVP-C5. Upon increasing the content of QPVP-C5 in the blend, the surface wettability and antimicrobial properties against *M. luteus* and *E. coli* were favored, indicating surface enrichment by pyridinium groups. For practical purposes, CMCAB/QPVP-C5 blends could be co-extruded with polyolefins, polystyrene, poly(methyl methacrylate), providing new materials with antimicrobial activity and good thermal stability, which can be used to manufacture daily items, like keyboards, doorknobs, handles, grips for supermarket

trolleys and baskets, with protection against microbial infections transmitted by contact.

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