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Surface cationized cellulose nanofibrils for the production of contact active antimicrobial surfaces

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ABSTRACT

In the last decade, a new fiber pretreatment has been proposed to make easy cellulose fibrillation into microfibrils. In this context, different surface cationized MFC was prepared by optimizing the experimental parameters for cellulose fibers pretreatment before fibrillation. All MFCs were characterized by conductometric titration to establish degree of substitution, field emission gun scanning electron microscopy (FEG-SEM), atomic force microscopy (AFM) and optical microscopy assessed the effect of pretreatment on the morphology of the ensuing MFCs. Antibacterial activities of neat and cationized MFC samples were investigated against Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*). The CATMFC sample at DS greater than 0.18 displayed promising results with antibacterial properties without any leaching of quaternary ammonium into the environment. This work proved the potential of cationic MFCs with specific DS for contact active antimicrobial surface applications in active food packaging, medical packaging or in health and cosmetic field.

1. Introduction

Since the mid of 80s, the emergence of resistant bacteria is considered as a major issue and an important threat to the public health as a result of the evolution of new infectious diseases (Feighner & Dashkevicz, 1987; Gilbert & Moore, 2005). The progressive reduction of the effectiveness of antimicrobial toward these resistant bacteria underlines the necessity (i) to evaluate the efficiency of available antimicrobial, (ii) the need to develop novel classes of antimicrobials, (iii) to limit their release in the environment which leads to the emergence of resistant bacteria (Magiorakos et al., 2012; Poverenov et al., 2013). Since 2000s, cationic compounds have emerged as promising candidates for further improvement as antimicrobial agents decreasing evolution of resistant strain. Among these cationic compounds, quaternary ammonium moieties-bearing molecules are widely used, since decades, as antiseptic and disinfectant (Grare et al., 2009), however, their release still limits their applications.

Meanwhile, new contact active antimicrobial surfaces have been developed to restrict the release of active molecule and subsequently the evolution of new resistant bacteria. When biobased and renewable materials are concerned, some successful studies can be quoted with chitosan based materials (Antunes, Moreira, Gaspar, & Correia, 2015; Zarei, Ebrahimiasl, & Jafarirad, 2014), active protein release (Cozzolino et al., 2013; Lavoine, Desloges, Sillard, & Bras, 2014; Li et al., 2014) and grafted fibers (Illergard, Rmling, Wagberg, & Ek, 2012; Österberg et al., 2013). Recently, high specific area microfibrils of cellulose (MFC) have been grafted with active molecules (Fernandes et al., 2013; Missoum, Sadocco, Causio, Belgacem, & Bras, 2014; Saini, Belgacem, Mendes, & Elegir, 2015). MFC are produced from cellulose fibers as a result of high shear mechanical fibrillation process. After its discovery in 80s by Turbak, Snyder, and Sandberg (1983), an exponential increase of research and application was recorded with the development of fiber pretreatments. With regard to the commercial production of MFC, the most common fiber pretreatments are enzymatic or oxidation, aiming at reducing the energy consumption during fibrillation (Isogai, Saito, & Fukuzumi, 2011; Lavoine, Desloges, Dufresne, & Bras, 2012; Siro & Plackett, 2010). Recently, a new cationization pretreatment has been developed, which, not only reduces the energy consumption for the production of MFC, but also adds new functionalization to the fibrils.

Various cationization agents, such as chlorocholine chloride (ClChCl) (Ho, Zimmermann, Hauert, & Caseri, 2011), Girard's reagent T ((2-hydrazinyl-2-oxoethyl) trimethyl azanium chloride)

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(Liimatainen, Suopajärvi, Sirviö, Hormi, & Niinimäki, 2014), and 2,3-epoxypropyl trimethylammonium chloride (EPTMAC) (Olszewska et al., 2011; Pei, Butchosa, Berglund, & Zhou, 2013) are mentioned in the literature. However, very few investigations dealing with the experimental optimization of such systems have been performed due to the novelty of these cationized MFCs. Cationic MFC demonstrates the potential to improve compatibility and homogeneous dispersion within polymer matrix (Kalia, Boufi, Celli, & Kango, 2014) and decreases energy consumption at the last step of mechanical disintegration process (Habibi, 2014). As a result of cationization, fibrils with highly swollen outer layer facilitate effective fibrillation in aqueous media (Olszewska et al., 2011).

In another study, the ability of cationic MFC to be used as dye removal from aqueous waste streams due to their high anionic dye adsorption capability was also examined. In addition, cellulose nanopapers prepared with that cationized MFCs displayed high tensile strength (ca. 200 MPa) and Young's modulus (ca. 10 GPa) despite high porosity (37–48%) as well as ultrahigh water absorbency (750 g/g) (Pei et al., 2013). Moreover, high flocculation capacity of cationized cellulose and MFC was confirmed over a wide range of pH for wastewater treatment (Liimatainen et al., 2014; Song, Zhang, Gan, Zhou, & Zhang, 2010; Yan, Tao, & Bangal, 2009). However, so far no study dealt with the utilization of cationic pretreatment for antimicrobial activity estimation.

Nevertheless, quaternization is also employed as a post treatment of nanofibrils for antimicrobial properties against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Andresen et al., 2007). Recently, Hua et al. investigated the cytocompatibility of trimethylammonium-modified MFC and proposed the potentiality of cationized MFC films in tissue engineering applications (Hua et al., 2014). However, post treatment imposes extra cost and also restricts its large scale application.

Therefore, this work first optimizes experimental conditions for fiber pretreatment considering the production of cationized microfibrillated cellulose to attain high degree of substitution (Fig. 1). The second part deals with the detailed investigation of antimicrobial release and quantification of the microbial growth inhibition by cationic MFC with promising results.

2. Materials and methods

2.1. Materials and chemical

Cellulose pulp JELUCEL® from oat is obtained from Jelu-Werk, Germany and used as supplied. Chemicals have been purchased from different suppliers (detailed in brackets): cationising agent 2,3-epoxypropyl trimethylammonium chloride (Sigma–Aldrich), sodium hydroxide (Roth, France), sodium thiosulphate anhydrate (Roth, France), sodium chloride (Roth, France), acetic acid (Chimie Plus, France), FiberCare® R cellulase (Novozyme, Sweden), nutrient agar (Roth, France) and nutrient Broth (Roth, France). *Bacillus subtilis* bacterial spores (Humeau, France), *Escherchia coli* and *Staphylococcus aureus* were bought in lyophilized form.

2.2. Preparation of Enzymatic MFC (EnzMFC)

MFC suspension was produced from cellulose pulp JELUCEL®, enzymatically pre-treated (during 3 h with cellulase, 0.1% (w/w) at 50 °C and under stirring at 200 rpm), and passed through a Masuko® (model number MKCA6-2) ultra-fine friction grinder at 2500 rpm. The prepared concentration was at 2% (w/w).

2.3. Fabrication of MFC by cationic pretreatment

Nanofibrillated cellulose was produced according to the following steps: (1) refining pre-step with PFI mill (PTA, France), (2) cationization pre-treatment step, (3) fibrillation of cellulose pulp by a mechanical treatment with ultra-fine friction grinder Supermass colloider (Model MKZA6-2, Disk model MKG-C 80, Masuko Sangyo Co., Ltd., Japan).

The cationization reaction was adapted from previous study (Pei et al., 2013) and was carried out in two different methods: diluted (5%) and concentrated (7.5%). Cationization with diluted method was carried out in three-necked round-bottomed flask, whereas in concentrated method cellulose was placed in plastic bag and immersed in water bath. Several conditions were tested, as shown in Table 1.

A two-step strategy was followed for cationization. In first step, cellulose was activated with sodium hydroxide (NaOH) at 65 °C for 30 min, followed by cationization at the same temperature for 5 h. Pretreated fibers were then washed several times with deionized water/ethanol to eliminate all the reactant traces before defibrillation. The never dried suspensions (2%, w/w) were then passed through supermass colloider Masuko® at 2500 rpm and the energy consumption for each formulation was studied.

2.4. Determination of degree of substitution by conductometric titration

The number of quaternary ammonium groups was estimated for pretreated fibers by conductometric titration of chloride ions with aqueous silver nitrate ($AgNO_3$), assuming the presence of one chloride counter ion per trimethylammonium group. Typically, cationized cellulose suspension giving 1 g of cellulose in dry basis was titrated with 0.01 M $AgNO_3$ (aq.) by adding approximately 0.5 mL in 30 s intervals. Degree of substitution (DS) was calculated on the basis of $AgNO_3$ volume as follows:

$$\text{Degree of substitution, DS} = \frac{n\text{AgNO}_3}{n_{\text{cellulose}}} \quad (1)$$

where $n_{\text{cellulose}}$ is the units of anhydroglucose present in 1 g of cellulose and $n\text{AgNO}_3$ is the number of mole of $AgNO_3$ present in the volume spent to titrate the cationized MFC.

2.5. Characterization of MFC

2.5.1. Optical microscopy

Photomicrographs of MFC suspensions were taken using optical microscopy (Zeiss Axio Vert.A1). Typically, a few drops of MFC suspension (0.3 wt.%) were placed on a standard microscopy slide and covered with a cover glass for observation. The pictures were taken at different scale and the most representative ones were selected among at least 10 pictures.

2.5.2. Morphological fiber analysis (MorFi)

Native cellulose, refined cellulose suspension, cationized cellulose samples, and fabricated MFCs after each pre-treatment were analyzed with MorFi (Techpap, France). The samples subjected to MorFi analyser were diluted with 0.3 wt.% MFC. In MorFi analysis cell, a digital camera and a software package for image analysis are integrated in order to automatically measure the dimension of the fibers in the suspension. It is designed to analyze diluted pulp suspensions and to measure different parameters such as fiber length and width, the content of fiber and fine elements. MorFi is programmed to consider fiber length values between 200 and 10,000 μm, width between 5 and 75 μm, fines length below 200 μm and width below 5 μm and fiber class of 200 μm.

2.5.3. Atomic force microscopy/scanning electron microscope/field emission gun-scanning electron microscope

AFM (Veeco NanoScope-V, USA) was done by placing a drop (~50 μl) of 0.01 wt.% MFC suspension samples on the clean mica