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New supramolecular metallo-terpyridine carboxymethyl cellulose derivatives with antimicrobial properties

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ABSTRACT

Preparation of a new water-soluble, cellulose derivative *via* a supramolecular route is presented. In a one-step procedure, carboxymethyl cellulose (CMC) was reacted with the $Cu(BF_4)_2$ complex of 4′-chloro[2,2′:6′,2″]terpyridine to generate the desired CMC–Cu^{II}–terpyridine derivative. This polymeric salt was characterized by elemental analysis, ultraviolet–visible spectroscopy (UV–visible), Fourier transform infrared (FTIR), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), rheological properties measurements, thermogravimetric analysis (TGA), dynamic mechanical thermal analysis (DMTA), and tensile strength properties testing. In addition, antimicrobial properties were demonstrated against Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus thermophilus*), Gram-negative bacteria (*Escherichia coli*), and yeast (*Saccharomyces cervisiae*). The minimum inhibitory concentration of the prepared metallo-terpyridine CMC derivative against the studied microorganisms ranged from 6 to 8 mg/L to achieve ≥90% of microbial growth inhibition.

1. Introduction

The practice of using cellulose for preparation of new polymeric derivatives has been on-going for more than a century. Carboxymethyl cellulose (CMC, **1**) is an important as well as very useful carboxylate-modified, ether derivative, consisting of two units: β-D-glucose and β-D-glucopyranose 2-*O*-(carboxymethyl) monosodium salts, which are connected *via* β-1,4-glycosidic bonds. Attachment of the carboxymethyl moieties occurs predominantly at the C-2 and C-6 glucose positions with a preference for C-6 *vs.* C-2 (Heinze & Pfeiffer, 1999). Its water solubility, rheological properties, non-toxicity, and polyelectrolyte nature favor its use in many diverse applications, such as food additives (Parvar, Tehrani, Razavi, & Koocheki, 2013), pharmaceutics (Chukwumezie, Wojcik, Malak, & Adeyeye, 2002), bone regeneration (Jiang, Li, Zhang, & Wang, 2009), adhesives (Kawamoto, 2003), textiles (Krizova & Wiener, 2013), pesticides (Nisar, Kumar, Shakil, Pankaj, & Parmar, 2009), detergents (Verraest, Peters, van Bekkum, & van Rosmalen, 1996), paper (Li, Liu, Xu, & Xu, 2010; Watanabe, Gondo, & Kitao, 2004), and ceramic binders (Khosrowshahi & Salem, 2011).

In spite of the numerous desirable properties of CMC, it still lacks resistance to microbial attack due to its polysaccharide nature and absence of antimicrobial functional groups. Different routes have been studied to impart CMC antimicrobial properties, such as: the addition of antimicrobial agents (Liu, Han, Zhang, Li, & Li, 2012; Ng & Jumaat, 2014; Sayanjali, Ghanbarzadeh, & Ghiassifar, 2011); metal nanoparticles (Hebeish, Hashem, El-Hady, & Sharaf, 2013; Malmiri, Tan, Rahman, & Osman, 2013; Percival et al., 2012; Valappil et al., 2013; Zhong, Oporto, Jaczynski, Tesfai, & Armstrong, 2013), antimicrobial polymers (Yu et al., 2013); and grafting with other moieties possessing antimicrobial activity (El-Sherbiny, Salama, & Sarhan, 2009).

Terpyridines are *N*-heterocycles that exhibit high binding affinity toward transition metal ions due to strong dπ–pπ* back-bonding of the metal to the pyridine rings and the chelate effect. These terpyridine metal complexes possess distinct photophysical, electrochemical, and magnetic properties and have been widely investigated (Schubert, Winter, & Newkome, 2011). Notably, antimicrobial properties of terpyridine metal complexes have been investigated (El Basel Hassanien, 2004; Kharadi, 2014;

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Kharat, Foroutannejad, & Khavasi, 2012; Naseri, Kharat, Banavand, Bakhoda, & Foroutannejad, 2012; Pandrala et al., 2013; Patel, Singh, Shukla, Gundla, & Chauhan, 2006; Patel, Parmar, Gandhi, & Thakkar, 2010; Patel, Joshi, & Patel, 2012a; Patel, Dosi, & Bhatt, 2012).

Terpyridine-containing polymers have also been investigated in which terpyridine ligands have been (1) incorporated into monomers that were subsequently polymerized, (2) attached as polymeric chain ends to be utilized in the formation of block copolymers, and (3) attached to polymeric backbone to generate new materials utilizing the available pendent terpyridine units that can be further transformed into metallo-supramolecular assemblies (Aamer & Tew, 2007; Breul et al., 2013; Chiper, Hoogenboom, & Schubert, 2009, 2010; Constable, 2007; Dobrawa, Ballester, Saha-Möller, & Würthner, 2006; Hassan, Moorefield, Elbatal, & Newkome, 2012; Hassan, Moorefield, Elbatal, & Newkome, et al., 2012; Köytepe, Demirel, & Seçkin, 2013; Köytepe, Demirel, Gültek, & Seçkin, 2013; Li, Guo, et al., 2013; Li, Zhu, et al., 2013; Maeda, Sakamoto, & Nishihara, 2013; Muronoi, Zhang, Higuchi, & Maki, 2013; Yang et al., 2013; Zhang, Zhang, & Chen, 2006); however, to the best of our knowledge, none of the terpyridine-containing metallopolymers have been investigated regarding their antimicrobial properties.

We have recently reported the preparation of terpyridine-modified cellulose nanocrystals *via* etherification of cellulose with 4′-chloro[2,2′:6′,2″]terpyridine (Cltpy) and subsequently examined their ability to assemble to metallo-supramolecular cellulose derivatives (Hassan, Moorefield, Elbatal, & Newkome, 2012; Hassan, Moorefield, Elbatal, & Newkome, et al., 2012). Herein, we report the preparation of water-soluble, terpyridine-based, supramolecular cellulose composite and demonstrate its utilitarian antimicrobial activity.

2. Experimental

2.1. Materials

Carboxymethyl cellulose (CMC) was obtained as a high viscosity grade (1000–1500 mPa for 4% in water at 25 °C) with DS of 0.93 as determined by the standard method (ASTM, 1961). 4′-Chloro[2,2′:6′,2″]terpyridine (Cltpy; assay 99%), $Cu(BF_4)_2{\cdot}6H_2O$ (assay 99%), NaOH, and absolute EtOH were obtained from Sigma-Aldrich and used, as received.

2.2. Preparation of CMC–Cu^{II}–terpyridine

The 4-Cl-terpyridine–$Cu(BF_4)_2$ [Cltpy–$Cu(BF_4)_2$] adduct was synthesized according to a previously published procedure (Wang et al., 2006). Reaction of Cltpy–$Cu(BF_4)_2$ (1.5 g, 6.82 mmol) with an aqueous solution of CMC (1 g in 150 mL of water) at 70 °C for 3 h generated the desired CMC–Cu^{II}tpyCl(BF_4) (**I**) along with a minor insoluble, cross-linked CMC–Cu^{II}–tpyCl (**II**), which was easily filtered. The filtrate contains the desired soluble CMC–Cu^{II}terpyCl(BF_4) (**I**) (Scheme 1), which was collected and purified by repeated precipitation and finally washing from/with an EtOH/water (7:3) mixture.

2.2.1. Characterization of CMC–Cu^{II}tpyCl(BF_4)

UV–visible spectra were recorded on UV-2450 Shimadzu spectrophotometer. The X-ray diffraction patterns were recorded using. The wave length of the Cu/$K\alpha$ radiation source was 0.154 nm and the spectra were obtained at a 30 mA with an accelerating voltage of 40 kV. A Perkin-Elmer Thermogravimetric analyzer was used to study thermal stability. The heating rate was set at 10 °C/min over a temperature range of 50–800 °C. Measurements were conducted under N_2 with a flow rate of 50 cm^3/min. XPS studies were performed using a Thermo Scientific K-ALPHA, XPS, England. Infrared spectra were obtained by using JASCO FTIR 800 E spectrometer. The samples were measured using the KBr disc technique. The elemental analysis was conducted on a Vario EI Elementar instrument. Degree-of-substitution (DS) of cellulose derivative was calculated from the nitrogen content as follows:

$$DS_x = \frac{M_c \times \%X}{100 \times M_x - \Delta M \times \%X}$$

where M_c is the molar mass of CMC monomeric unit at DS = 1; $\%X$ is the element or group; M_x is the molar mass of the element or the group; $\Delta M = M_s - M_L$, where, M_s is the molar mass of the substituent; M_L is the molar mass of the leaving group; and ΔM is the increase in molar mass of the monomeric unit at DS = 1.

Rheology of an aqueous solution (0.5 wt %) of CMC or CMC–Cu^{II}tpyCl(BF_4) (**I**) was studied using Anton Paar Rheometer (Anton Paar, Austria); a 2.5 cm plate and plate system was used at 25 ± 0.1 °C and a shear rate from 1 to 500 min^{-1}. Films were casted from aqueous solution (0.5 wt%) of CMC and **I**; each was dried in an oven under air circulation at 40 °C for 12 h. After drying, the films were conditioned at 50% relative humidity and 25 °C for 48 h before testing. Dynamic mechanical thermal properties of the films were measured using the same machine in tensile mode at a frequency of 1 Hz, strain of 0.1%, and at the heating rate of 3 °C/min over the temperature range of 25 to 200 °C. Tensile properties of the films were measured using a Lloyd universal testing machine (Lloyd, England) at crosshead speed of 2 mm/min using a load cell of 0.1 kN. The width of the films was 1 cm and the gauge length was 4 cm.

The antimicrobial activity of CMC and CMC–CltpyCu^{II}(BF_4) was evaluated by minimal inhibitory concentrations test (MIC). Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus thermophilus*), Gram-negative bacteria (*Escherichia coli*), as well as yeast (*Saccharomyces cervisiae*) were used, as test organisms. A pre-culture of bacteria and yeast was grown in Tryptic Soy Broth medium with 0.6% yeast extract overnight at 37 or 30 °C for bacteria and yeast, respectively. The inoculums were prepared by diluting an overnight culture grown in Tryptic Soy Broth. Each microorganism culture was exposed to different concentrations (1, 2, 4, 6, and 8 mg/mL) of the modified CMC–CltpyCu^{II}(BF_4) and incubated at 37 or 30 °C with shaking at 140 rpm. After a 24 h incubation, a series of solutions were prepared by addition of 1 mL of each culture to 9 mL of sterile 0.3 mM phosphate buffer (pH 6.8), followed by seeding 100 μL of each culture solution onto an agar plate. The plates were incubated at 37 or 30 °C, according to the type of microorganism for 24 h and the surviving cells counted. The antimicrobial activity was expressed as a reduction of the bacterial colonies after contact with the test specimen and compared to the number of bacterial colonies from the control. The percentage reduction (inhibition) was calculated using the following equation:

$$\text{reduction} = \frac{B - A}{B} \times 100$$

where, A are the surviving cells (CFU—colony forming units) for the plates containing the treated substrate and B are the surviving cells from the control.

3. Results and discussion

3.1. Preparation and characterization of CMC-CltpyCu^{II}(BF_4)

Reaction between CMC and $Cu(BF_4)_2$-tpyCl created a pentacoordinate complex involving the carboxylate groups of CMC (Wang et al., 2006); two main reactions occur leading to the formation of a complex between at least one carboxylic group of CMC chain and CMC-CltpyCu(BF_4) (Scheme 1; product **I**) or the complex between two carboxylic groups of two different CMC chains and