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Choline chloride–thiourea, a deep eutectic solvent for the production of chitin nanofibers

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ABSTRACT

Deep eutectic solvents (DESs) consisting of the mixtures of choline halide (chloride/bromide)–urea and choline chloride–thiourea were used as solvents to prepare α-chitin nanofibers (CNFs). CNFs of diameter 20–30 nm could be obtained using the DESs comprising of the mixture of choline chloride and thiourea (CCT 1:2); however, NFs could not be obtained using the DESs having urea (CCU 1:2) as hydrogen bond donor. The physicochemical properties of thus obtained NFs were compared with those obtained using a couple of imidazolium based ionic liquids namely, 1-butyl-3-methylimidazolium hydrogen sulphate [(Bmim)HSO_4] and 1-methylimidazolium hydrogen sulphate [(Hmim)HSO_4] as well as choline based bio-ILs namely, choline hydrogen sulphate [(Chol)HSO_4] and choline acrylate. The CNFs obtained using the DES as a solvent were used to prepare calcium alginate bio-nanocomposite gel beads having enhanced elasticity in comparison to Ca-alginate beads. The bio-nanocomposite gel beads thus obtained were used to study slow release of 5-fluorouracil, an anticancer drug.

1. Introduction

Nanofibers (NFs) are extensively used in nanotechnology for the preparation of functional nanocomposites (Huang, Zhang, Kotaki, & Ramakrishna, 2003). The widely used technique to produce NFs is the electrospinning process, which uses an electrical charge to draw very fine fibers from polymer solutions (Subbiah, Bhat, Tock, & Parameswaran Ramkumar, 2005). NFs are also useful in various biomedical applications and hence substantial efforts are being made to develop biodegradable polymer scaffolds suitable for tissue engineering applications (How, Guidoin, & Young, 1992; Vacanti & Vacanti, 1997). Matthews, Wnek, Simpson, and Bowlin (2002) have attempted to prepare tissue-engineering scaffolds composed of collagen nanofibers by optimizing various parameters of electrospinning process (Matthews et al., 2002). Cellulose NFs were prepared directly from wood by Abe, Iwamoto, and Yano (2007).

Chitin, the (1–4)-2-acetamido-2-deoxy-β-D-glucan, is industrially produced from marine resources (Muzzarelli, 2012; Muzzarelli et al., 2012). Chitin and chitosan activate the macrophages, and are mucoadhesive, antimicrobial, biodegradable and nontoxic and therefore they are widely used for the repair of wounded human tissues (Muzzarelli, 2009). Chitin has been processed in the form of nanofibers (CNFs) or nanocrystals (CNC) by several research groups by employing various techniques, such as acid hydrolysis (Morin & Dufresne, 2002; Lu, Weng, & Zhang, 2004; Goodrich & Winter, 2007), TEMPO mediated oxidation (Fan, Saito, & Isogai, 2008a, 2008b), ultrasonication (Zhao, Feng, & Gao, 2007) and electrospinning (Jayakumar, Prabaharan, Nair, & Tamura, 2010). More recently, Isogai et al. (2012) have reported comparative characterization of aqueous dispersions and cast films of different chitin nano whiskers (Fan, Fukuzumi, Saito, & Isogai, 2012). Nanofibrillation efficiency of α-chitin in various organic and inorganic acids by varying the pH and ionic strength was reported (Qi et al., 2013). CNFs having good dispersibility in 2,2,2-trifluoro ethanol and preparation of its nanocomposite with polycaprolactone is reported (Ji, Wolfe, Rodriguez, & Bowlin, 2012).

Ionic liquids (ILs) are low melting point salts that form liquids at temperatures below the boiling point of water. Applications of ILs in carbohydrate and polysaccharide chemistry are increasing and they are being used to prepare a number of new compounds (El. Seoud, Koschella, Fidale, Dorn, & Heinze, 2007). Biomedical exploitation of chitin is currently under way with the aid of ionic liquids (Muzzarelli, 2011). Attempts are also made to process chitin in NF form using ILs, e.g., 1-ethyl-3-methylimidazolium acetate was used to dissolve crustacean shells and highly pure high molecular weight chitin powder as well as fibers was recovered (Qin, Lu, Sun, & Rogers, 2010; Barber, Griggs, Bonner, & Rogers, 2013). Kadokawa,

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Takegawa, Mine, and Prasad (2011) have demonstrated application of 1-ethyl-3-methylimidazolium bromide for the preparation of chitin nanowhiskers. Deep eutectic solvents (DES) are fluids obtained by heating two or more compounds capable of self-association through hydrogen bond interactions and have lower melting points in comparison to each of the individual components (Zhang, Vigier, de Oliveira, Royer, & Jérôme, 2012). Abbott, Boothby, Capper, Davies, and Rasheed (2004) for the first time presented DESs as suitable alternative solvents to ILs. DES has similar physicochemical properties in comparison to ILs but they are considered different from ILs mainly because (a) they do not entirely consist of ionic species and (b) they can be prepared from non-ionic species as well unlike the ILs (Zhao & Qu, 2013). Further, the DESs are more advantageous due to their cheaper cost and environmentally friendlier nature. Owing to these remarkable advantages, DESs are now of growing interest in many fields of research, e.g., catalysis, organic syntheses, dissolution media, extraction processes, electrochemistry and material chemistry (Zhang et al., 2012). We have demonstrated recently suitability of few bio-ILs and DESs for the solubilization of natural polymers such as DNA and α-chitin (Mukesh, Mondal, Sharma, & Prasad, 2013; Mondal, Sharma, Mukesh, Gupta, & Prasad, 2013; Sharma, Mukesh, Dibyendu, & Prasad, 2013).

Herewith, we report production of chitin nanofibers using a deep eutectic solvent (choline chloride–thiourea) and application of the nanofibers as reinforcement fillers for calcium alginate beads having ability to release 5-fluorourascil, an anticancer drug. The physicochemical properties of the NFs further compared with those prepared using a couple of imidazolium based ionic liquids namely 1-butyl-3-methylimidazolium hydrogen sulphate ($BmimHSO_4$) and 1-methylimidazolium hydrogen sulphate ($HmimHSO_4$) as well as choline based bio-ILs namely, choline hydrogen sulphate ($Chol.HSO_4$) and choline acrylate.

2. Materials and methods

2.1. Materials

Choline chloride, 1-methylimidazole, 5-fluorouracil and chitin powder obtained from crab shells were purchased from TCI Fine chemicals, Tokyo, Japan. The degree of polymerization of chitin from the origins was reported to be ca. 2000–4000 (Hasegawa, Isogai, & Onabe, 1994; Kurita, 2001). The degree of acetylation of the chitin sample was estimated by elemental analyses data and found to be 94.1%, which was in good agreement with that of standard chitin (Guinesi & Cavalheiro, 2006). The FT-IR spectra of the sample showed vibrational bands at 1662 cm^{-1} and 1631 cm^{-1} in the amide I region characteristic of α-chitin (Cárdenas, Cabrera, Taboada, & Miranda, 2004). The band at 1540 cm^{-1} corresponds to protein absorption, which are absent in FT-IR spectrum indicating absence of protein impurities in the sample. 1-Butyl-3-methylimidazolium chloride was purchased from Merck & Co., Germany. Sodium alginate was purchased from Sigma–Aldrich Chemical Co., USA. Thiourea, acrylic acid (AA), and $CaCl_2$ were purchased from S.D. Fine chemicals, Mumbai, India. All chemicals were of analytical grade and were used as received without further purification.

2.2. Measurements

Powder X-ray diffraction patterns were recorded at 298 K on a Phillips X'pert MPD system using CuK_α radiation ($\lambda = 0.15405$ nm) with 2θ range from 10° to 80° at a scan speed of 0.1° s^{-1}. The dry samples were placed on carbon coated copper grids (300 mesh sizes) and the transmission electron microscopic (TEM) images of thus prepared samples were obtained using a JEOL transmission electron microscope (Model JEM 2100, Japan) operated at accelerating voltage of 120 kV. The dry samples were dispersed in acetone and coated on aluminum stubs and evaporated to dryness followed by recording of their SEM images on an LEO 1430 VP instrument employing accelerating voltage of 18 kV. FT-IR analyses were carried out on a Perkin Elmer Spectrum GX, FTIR System, USA, by taking 2.0 mg of sample in 600 mg of KBr. All spectra were averages of two counts with 10 scans at a resolution of 5 cm^{-1}. ^1H NMR of the ILs and DES were recorded on a Bruker Avance-II, 500 MHz spectrometer. Linear viscoelastic properties (controlled deformation mode with 0.01% strain of Ca-Alg beads and Ca-Alg/CNF bio-nanocomposite beads were carried out on an Anton Paar, Physica MCR 301 rheometer, USA, using parallel plate PP50/P-PTD200 geometry (50 mm diameter; 0.1 mm gap) operating in dynamic mode. All the dynamic rheological data were checked as a function of strain amplitude to ensure that the measurements were performed in the linear viscoelastic region. The UV–vis absorption spectra of acidic ionic liquids were recorded on a Varian CARY 500 UV–Vis–NIR Spectrophotometer, USA. CNF solutions in distilled water were used for AFM sample preparation. Appropriately diluted nanofibers (0.05 mg/mL) solutions were deposited on freshly cleaved mica foil. After 2 min the solution was drained off and dried by nitrogen gas. The mica foil was then kept in dust free $CaCl_2$ desiccators for five days and then was used for AFM analysis. The AFM measurements were performed in the semi contact mode using an Ntegra Aura (Nt-Mdt, Russia) instrument at room temperature in air. The height of images was recorded from different area of each sample. The software used for image analysis was "Nova". Elemental analyses were carried out on a Perkin-Elmer CHNS analyser.

2.3. Synthesis of DESs and acidic ionic liquids

In a typical reaction for the synthesis of 1-butyl-3-methylimidazolium hydrogen sulphate [(Bmim)HSO_4], [(Bmim)]Cl (5 g, 0.0286 mol) was added into 20 mL dichloromethane in a round-bottom flask followed by drop wise addition of one equivalent of sulphuric acid (98%) (1.5 mL, 0.0286 mol) for 10 min at room temperature. The reaction mixture was refluxed for 24 h at 70 °C and the resulting IL was separated out, washed with ethyl acetate two times and dried at 70 °C for 6 h under vacuum. Similar metathesis reaction was carried out for the synthesis of the other two ILs, *viz*., choline hydrogen sulphate [(Chol)HSO_4], methyl imidazolium hydrogen sulphate [(Hmim)HSO_4] and choline acrylate. The structures were confirmed by ^1H NMR and mass spectrometry (ESI-MS).

DESs were prepared following the method described by Abbott et al. (2004). In a typical reaction, both the hydrogen bond donor (HBD) and acceptor (HBA) molecules were heated at 50 °C (reactions where urea was used as HBD) and 70 °C (reactions where thiourea was used as HBD) with constant stirring at optimized mole ratios as shown in Table 1 under argon atmosphere until homogenous and colorless liquids were formed (Supporting Fig. S1).

2.4. Preparation of α-chitin nanofibers

Pure chitin powder (at optimized concentration of 10%, w/w) was dispersed separately in DESs and acidic ILs followed by stirring at 500 rpm at 100 °C for different duration of time as shown in Table 1. The gel like materials thus obtained was diluted by adding 10 mL of distilled water. The dispersed chitin particles (Fig. 1) were collected after centrifugation at 10,000 rpm for 10 min followed by washing with distilled water to make them free from acid (Step 1). The acid free chitin was further dispersed in distilled water (30 mL) and the solution was ultra sonicated using an ultra sonication rod