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Synthesis of the ketimine of chitosan and 4,6-diacetylresorcinol, and study of the catalase-like activity of its copper chelate

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ABSTRACT

In this study, a new chitosan derivative (ketimine) was synthesized by condensation of chitosan with 4,6-diacetylresorcinol (DAR) at heterogeneous medium. The ketimine derivative of chitosan (DAR-chitosan) was characterized by elemental (C, H, N), spectral (DR-UV–vis and FT-IR spectroscopy), structural (powder XRD), and morphological (SEM) analyses. The degree of substitution (DS) of DAR-chitosan was evaluated by elemental analysis and ^{13}C CP-MAS NMR spectroscopy and found to be around 12%. The copper (II) metal complex of DAR-chitosan was prepared and characterized by FT-IR, DR-UV–vis and inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Thermal behaviors of the synthesized compounds were investigated by DSC and TG-DTG-DTA analysis. The catalytic activity of copper (II) complex of chitosan derivative (DAR-chitosan-Cu) was investigated on hydrogen peroxide decomposition. The copper chelate showed high efficiency (over 80%) towards the decomposition of hydrogen peroxide as heterogeneous catalyst.

1. Introduction

Chitosan is a natural, cationic amino polysaccharide co-polymer of glucosamine and *N*-acetylglucasamine, obtained by the alkaline, partial *N*-deacetylation of chitin (Muzzarelli, 1977). Chitosan has been considered as a non-toxic, biodegradable, biocompatible and environmentally friendly material with many superior properties (Jigar & Sinha, 2007; Muzzarelli & Ilari, 1994). This biopolymer has applications ranging from artificial skin, photography, cosmetics, food and nutrition, ophthalmology and wastewater treatment (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). In addition, chitosan has received significant interest for removal of heavy metal ions due to its good metal-binding capacities and easy reuse, as well as its relatively low cost compared with activated carbon and its possible biodegradability after usage (Muzzarelli, 2011; No & Meyers, 2000).

In the last years there have been many studies interested in chemical modification of chitosan and its derivatives to enhance their properties and consequently expand their potential applications (Heras, Rodriguez, Ramos, & Agullo, 2001; Terada et al., 1999; Yalçınkaya, Demetgül, Timur, & Çolak, 2010). Hence, functionalization of chitosan provided catalysts for oxidation of alkyl benzene (Chang, Wang, & Su, 2002), cyclopropanation of olefins (Sun, Xia, & Wang, 2002), Suzuki and Heck reactions (Hardy, Hubert, Macquarrie, & Wilson, 2004).

The presence of primary amine in the polymeric chain of chitosan leads to the possibility of a several chemical modifications, including the preparation of Schiff bases (Moore & Roberts, 1981; Muzzarelli et al., 1988). It is well known that, the diketone; 4,6-diacetylresorcinol (DAR) serves as a starting material for the generation of multidentate symmetrical Schiff bases (Shebl, 2009). In this sense the modification of chitosan with aldehydes and ketones to produce Schiff bases may result in a potentially complexing material for metallic species with potential analytical and environmental applications (Hardy et al., 2004; Wang, Sun, & Xia, 2003).

It is well known that, chitosan can be chemically modified at very high degrees of substitution by homogenous reactions but at low levels by heterogeneous reactions (typically up to DS > 0.3). Although very high DS can be achieved when chitosan is dissolved in acidic solutions, many studies show that functionalization, even under mild conditions, dramatically reduces the molecular weight of the chitosan (Macquarrie & Hardy, 2005).

It has been known for about a century that the decomposition of H_2O_2 to H_2O and O_2 is considerably accelerated by many metal ions (Haber & Weiss, 1934; Kremer, 1985). The decomposition of hydrogen peroxide has been used as a model reaction for the investigation of the catalytic activity of various metal complexes and has also been studied as a catalase model. The catalase like properties of copper (II) complexes has also been investigated, but reports on this activity of copper (II)-containing systems reported are relatively

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scarce (Gao, Martell, & Reibenspies, 2003). The difference in reactivity of Cu(II) complexes towards H_2O_2 is due to the change in the redox potential of Cu(II) ions as a result of ligation with different ligands (Ozawa, Hanaki, Onodera, Kasai, & Matsushima, 1991).

The development of heterogeneous catalysts has become a major area of research recently, as the potential advantages like easy of separation, recovery, and reuse of catalysts, and the clean separation of product from the reaction mixture (Macquarrie & Hardy, 2005).

This study is aimed to synthesize of ketimine derivative of chitosan by condensation with 4,6-diacetylresorcinol (DAR) under heterogeneous reaction medium. Also, is aimed to prepare the metal complex (Cu^{2+}) of chitosan derivative and to investigate the catalytic activity of the chelate towards to hydrogen peroxide decomposition reaction.

2. Materials and methods

2.1. Instrumentation

Infrared (IR) spectra were recorded on a Perkin Elmer RX-1 FT-IR spectrophotometer using KBr pellets (4400–400 cm^{-1}). Room temperature diffuse reflectance spectra in the ultra- violet and visible region (DR-UV–vis) were recorded on a Varian Cary 100 model UV-Vis spectrophotometer. Elemental analyses were carried out with LECO-CHNS-932. The surface morphology of chitosan and derivatives were examined by scanning electron microscope (SEM). The samples observed by using a JEOL JSM 5500LV Scanning Electron Microscope with double sided carbon tape at an accelerating voltage of 5 kV. Metal analysis was carried out by ICP-AES (Varian model-Liberty Series II) in a solution prepared by decomposition of the complex with HNO_3 followed by dilution with bidistilled water. Differential scanning calorimetry (DSC) analyses were carried out by Perkin Elmer Pyris. The samples were heated from 40 °C to 350 °C at a heating rate of 10 °C min^{-1}. Thermogravimetric analyses (TG-DTA) of the samples were performed with DuPont 951 thermal analyzer under air atmosphere at a heating rate of 10 °C min^{-1}. X-ray diffraction spectrometry (XRD-powder) was obtained using a Rigaku System RadB X-Ray Diffractometer, using monochromated Cu Kα radiation in the range 2–40° (2θ), at 25 °C.

2.2. Materials

Chitosan with high molecular weight was purchased from Aldrich (Cat. number 41941-9, >75% deacetylated, Brookfield viscosity 800 cps). 4,6-Diacetylresorcinol (DAR) was used as crosslinking agent and was prepared by following literature procedure (Abou-Hussein, 2010). Cu^{2+} ion was used as its acetate salt for Cu(II) complex of crosslinked chitosan derivative. Working solutions of hydrogen peroxide were prepared weekly by volumetric dilution of 30% H_2O_2 (Merck) and were standardized daily by titration with potassium permanganate. All other chemicals were analytically pure and used without further purification.

2.3. Synthesis of ketimine derivative of chitosan (DAR-chitosan)

Ketimine derivative of chitosan (DAR-chitosan) ligand was prepared in two steps. The first step was the formation of 4,6-diacetylresorcinol (DAR) by acetylation of resorcinol (Emara, Tawab, El-ghamry, & Elsabee, 2011). The second step was the condensation of 4,6-diacetylresorciol (DAR) with chitosan. The crosslinking agent DAR (4.4 mmol) dissolved in methanol (50 dm^3) was added dropwise to the suspension of swollen 2.0 g of chitosan powder (8.8 mmol of glucosamine residue) in methanol with magnetic stirring. The mixture was refluxed for 24 h, which resulted in a yellow-colored compound. It was then decanted and thoroughly washed with methanol to remove any unreacted crosslinking agent and then dried in vacuum at 60 °C to give DAR-chitosan as a powder in yellow color (Scheme 1).

2.4. Synthesis of Cu(II) complex of DAR-chitosan (DAR-chitosan-Cu)

1.05 g of DAR-chitosan was suspended in methanol and stirred at 60 °C and then a hot solution of 0.50 g of $Cu(CH_3COO)_2{\cdot}H_2O$ in methanol (20 ml) was added drop wise. The mixture was stirred and refluxed for 24 h. The complex was filtered through a Gooch-3 filter and washed with methanol, water, dimethylsulfoxide (DMSO) and dimethylformamide (DMF) to remove the adsorbed metal ions, then dried in the vacuum oven (Scheme 2).

Yield: 1.10 g (78%); analytical data for DAR-chitosan-Cu: 6% Cu; color: green.

2.5. Catalase-like activity studies

The decomposition of H_2O_2 catalyzed by DAR-chitosan-Cu(II) complex can be monitored by titrating the undecomposed H_2O_2 with standard $KMnO_4$ solution (0.01 M). The chosen concentration of H_2O_2 was 3.5×10^{-2} M. In addition, the chosen amount range of catalyst was from 0.01 mmol to 0.1 mmol Cu(II) at a constant concentration of H_2O_2, pH and temperature.

The procedure for a study as follows; 50 mg of DAR-chitosan-Cu(II) complex (0.1 mmol Cu) loaded in a flask containing a 10 ml of 3.5×10^{-2} M hydrogen peroxide solution in aqueous phosphate buffer pH 6.86. Then, the reaction flask was thermostatted to 25 °C under constant stirring. Finally, the extent of hydrogen peroxide decomposed at different intervals of time (each 10 min; from 0 to 60 min,) was estimated by taking 2.5 ml aliquot of reaction mixture and titrating it with 0.01 M $KMnO_4$ in the presence of 0.01 M H_2SO_4. The procedure was repeated at different amount of DAR-chitosan-Cu(II). The reusability of the catalyst was also evaluated. After each experiment, the catalyst was separated from the reaction mixture by simple filtration. And also, the catalyst was washed with water and dried carefully before using in the subsequent run.

On the other hand, the reference experiments were operated by using CS and DAR-chitosan as catalysts.

3. Results and discussion

3.1. Solubility

Solubility of chitosan is related to the ionic concentration, pH, the distribution of acetyl groups along the chain, the intra-chain H bonds involving the hydroxyl groups and the molecular weight. It is well known that, solubilization of chitosan occurs by protonation of the $-NH_2$ group on the C-2 position of the D-glucosamine repeat unit, whereby the polysaccharide is converted to a polyelectrolyte in acidic media (Rinaudo, 2006).

The synthesized crosslinked-chitosan derivative (DAR-chitosan) was insoluble in aqueous solutions of organic acids and common solvents like acetone, ethanol, toluene, dichloromethane and DMF. This case can be explained by the decrease in number of $-NH_2$ groups of chitosan due to the condensation of carbonyl groups of DAR with these primary amino groups (Scheme 1). Also, the DAR-chitosan-Cu complex was insoluble in aqueous solutions of organic acids and other dilute acid solutions. Hence, this property of the complexes is important for easy separation from the reaction mixture while used as catalyst in the H_2O_2 decomposition reactions.