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Novel method for preparation of β-cyclodextrin/grafted chitosan and it's application

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Abstract

A novel technique for preparation of β-cyclodextrin-grafted chitosan was carried out by reacting β-cyclodextrin citrate (β-CD citrate) with chitosan. β-Cyclodextrin citrate was synthesis by esterifying β-cyclodextrin (β-CD) with citric acid (CA) in presence or absence of sodium hypophosphite as a catalyst in a semidry process. Different factors affecting preparation of β-CD citrates were studied to obtain β-CD citrate with high carboxyl content, such factors include reaction temperature, citric acid concentration, material to liquor ratio and duration. β-Cyclodextrin/grafted chitosan was prepared by coupling β-CD citrate with chitosan dissolved in different formic acid solutions having different concentrations. The reacting ingredients were subjected to various reaction conditions to attain the optimum condition. β-Cyclodextrin/grafted chitosan were evaluated by measuring the nitrogen content of both chitosan and grafted chitosan. Chitosan and β-cyclodextrin/grafted chitosan, having different molecular weights, were evaluated as antimicrobial agents for different microorganisms such as, *Bacillus Megaterium, Pseudomonas Fragi, Bacillus Cereus Staphylococcus Aureus, Escherichia Ecoli* and *Aeromonas hydra.*

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides. They are produced by enzymatic degradation of starch and were first obtained by Villies 1891. There are three main types of cyclodextrin,α, β and γ of six, seven and eight cyclic maltose, respectively (Kobayashi et al., 1981; Cox et al., 1984; Bender and Komiyama, 1978; Deratani and Poepping, 1995; Seo et al., 1987; Mizobuchi et al., 1980). The cyclodextrin consists of tours like macrocyclic ring. All hydroxyl groups are located at the top and bottom of the tours. Thus the hydrophobic cavity of cyclodextrins is capable of including a variety of hydrophobic compounds via host-guest complexation (Takashima et al., 2004). This property has been extensively exploited in the past to change physiocopharmacetical properties of lipophilic drugs such as water solubility, bioavailabilty, improved stability and effectiveness (Sortino et al., 2001).

Many attempts to utilize CD and CD derivatives in textile applications were carried out in the last decade (Szejtli, & Jozsef 2003; Buschmann, Knittel, & Schollmeyer., 2001). This was brought about by the recognition that the inclusion complex formation capability of CD can be applied to different area of applications such as deodorant, aroma, antimicrobial, insect repellent, mite repellent finishes that have recently become popular and in treating effluents.

Chitin is one of the most abundant naturally occurring polysaccharide next to cellulose. Chitin consists mainly of β-(1–4)-2-acetamido-2-deoxy-D-glucose units. Despite much recent research into its utilization, its tightly intermolecular hydrogen bonding and poor solubility to common organic solvents have so far prevented widespread utilization of chitin (George, 1992). Chitosan is N-deacetylated form of chitin that is obtained by alkaline treatment of chitin (50% of aqueous NaOH) at high temperature. Chitosan and its derivatives have become useful polysaccharides in the biomedical area because of its biocompatible, biodegradable, and non-toxic properties (Lee et al., 1997).

The antimicrobial and antifungal activities of chitosan and chitosan derivatives (Devlieghere, Vermeulen, & Debevere, 2004; Lim, Sang-Hoon; Hudson, &Samuel, 2004a,b) have been described, since chitosan inhibits the growth of a wide variety of bacteria and fungi. Moreover, chitosan has several advantages over other types of disinfectants, that is, it possesses a higher antibacterial activity, a broader spectra of activity, a higher killing rate, and lower toxicity toward mammalian cells.

Many attempts were carried out to prepare cyclodextrin-grafted chitosan in the literature. Zhang et al. prepared two new adsorbents by reacting of β-CD and sulfonated β-CD with

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epoxy-activated chitosan and chitosan, respectively (Zhang, Wang, & Yi, 2004). Wu Wen-Teng et al. immobilized β-CD to chitosan beads by crosslinking with1, 6-hexamethylene diisocyanate (Chiu, Chung, Giridhar, & WuChiu, 2004). Michel Morcellet studied the reaction of cyclodextrin monochlorotriazinyl derivative with chitosan (Martel et al., 2001), and Farusaki et al. (Furusaki et al., 1996) coupled carboxymethyl derivative of β-CD to chitosan.

In our pervious work, β-CD/grafted chitosan was prepared by graft copolymerization of β-CD itaconate onto chitosan using ceric ammonium nitrate as a redox initiation system (Gaffar et al., 2004). Cyclodextrin itaconate has been prepared by esterifying β-CD with itaconic acid using a semidry process. As continuity, efforts are made in this work to prepare cyclodextrin/grafted chitosan with high CD-content meanwhile it is easily and completely soluble in organic acid solutions. This was achieved by reacting of β-CD with citric acid at different reaction conditions to prepare β-CD citrate, as a reactive CD, of high carboxyl content. β-CD citrate was allowed to react with chitosan of three different molecular weights at various reaction conditions.

2. Experimental

2.1. Materials

β-Cyclodextrin was kindly supplied by Cerestar Co. (USA). Chitosan, having three levels of molecular weight, e.g. 50,000; 30,000; and 1500 was purchased Korea Chitosan Co., Ltd and other suppliers. Citric acid (CA), sodium hypophosphite monohydrate (SHP), formic acid, and sodium hydroxide were laboratory grade chemicals.

Bacillus megaterium 744 were obtained in lyophilized form, from Northen Regional Research Center, Illionion (USA). While *Pseudomonas* fragi NRRL B-727 was obtained from National Center for Agricultural Utilization Research (USA).

Bacillus cereus ATCC 11778 was obtained from American type culture collection (ATCC) (USA). While strain *Staphylococcus aureus* (No. 315) was obtained from US Food Drug Administration (FAD) Microbiology Lab (USA).

Tryptone Soya Broth and Tryptone Soya media were obtained from Oxoid Ltd, UK

2.2. Synthesis of β-cyclodextrin citrate

β-Cyclodextrin citrate (β-CD citrate) was prepared using a semidry reaction method by mixing of 2 g of β-CD with definite amount of water containing different citric acid concentrations (1–4 mole/1 g CD) in presence and absence of SHP. The reaction mixture was allowed to react in a circulating air oven at different reaction temperatures (80–140 °C) for specific times. The cured samples were purified by washing with isopropanol using a soxhlet for 6 h in order to remove unreacted components as well as any soluble fragments or by-products, followed by drying at 60 °C for 24 h. After drying, the β-CD citrate was kept over P_2O_5 for at least 48 h before analysis.

2.3. Preparation of β-cyclodextrin/grafted chitosan

Linking of β-CD citrate onto chitosan was taken place by reacting the pendant free carboxyl groups of β-CD citrate with the amino groups of chitosan. A definite volume of water containing different β-CD citrate concentrations was introduced into a solution containing chitosan dissolved in different formic acid concentrations (0–0.4 ml/1 g chitosan). The reaction mixture was then magnetically stirred and heated at different reaction temperatures (80–140 °C) for 3 h using different material-to-liquor ratios (1:10–1:25). At the end of the reaction, the products were precipitated by adding 100 ml of NaOH solution (0.2 N). The samples were thoroughly washed with distilled water till neutral (pH 7) to ensure the removal of unreacted β-cyclodextrin citrate. Finally, the samples were washed with acetone and oven dried at 60 °C for 24 h.

2.4. Antibacterial spectrum

Tryptone soya agar and broth were used for estimation the antibacterial spectrum of chitosan and cyclodextin/grafted chitosan, having three different molecular weights, on several spoilage and pathogen strains namely *B. cereus B. megaterium, Pseudomonas fragi, S. aureus, Aeromonas Hydra, and Escherichia coli*

Chitosan and cyclodextin/grafted chitosan samples of different concentrations (12.5–100 mg) were added prior pouring. All treatments were incubated at 37 °C for 24 h. All incubated media were visually observed to find out whether the strain had grown or not. Besides, the minimum inhibition concentration (MIC) of each strain was determined.

2.5. Testing and analysis

The carboxyl content of β-CD citrate was determined using acid base titration method according to a reported method (Yang & Wang, 2000).

The grafting efficiency of β-CD citrate onto chitosan was calculated mathematically via determining the nitrogen % of chitosan before and after modification according to Kjeldal method (Vogel, 1966).

The minimal inhibition concentration (MIC) of bacteriocin was qualitatively determined for several bacterial strains according to the reported method (Davidson & Parish, 1989).

3. Results and discussion

3.1. Tentative mechanism

Esterification of polysaccharides (e.g. cellulose (El-Tahlawy, 1999; Voncina & Le Marechal, 2005), starch (Xueju & Qiang, 2004)) can be prepared by the reaction of polycarboxylic acid with hydroxyl groups of these polymers in the presence of alkaline or amphoteric catalyst under curing conditions via reactive cyclic anhydride intermediate mechanism.

Preparation of β-CD citrate was carried out by the reaction of β-CD with citric acid, in presence and absence of SHP, via the