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Modification of chitosan derivatives of environmental and biological interest: A green chemistry approach

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ABSTRACT

Chitosan is a non-toxic polyaminosaccharide that is available in a variety of useful forms, and its chemical and biological properties make it a very attractive biomaterial that could be used in a wide variety of medicinal applications. This work focuses on the preparation of different chitosan derivatives by treatment with ethyl cellulose, cellulose triacetate and different carbohydrates in both neutral and slightly acidic media. It also addresses modification with glycidyltrimethyl ammonium chloride, phthalic anhydride and succinic acid derivatives. The obtained derivatives were crosslinked with glutaraldehyde. Thermo-gravimetric (TGA) and FT-IR spectroscopic analyses and electron scanning microscopy (SEM) were used to characterize the obtained products and demonstrate the success of the chitosan-modification process. The obtained products were tested for their ability to uptake transition metal ions from aqueous solutions, and their ion-uptake efficiency was determined with the aid of the ICP-AES technique. The bioactivity of some selected products was tested to study the effect of their concentrations on selected microorganisms. *Burkholderia cepaci*, *Aspergillus niger*, and *Candida albicans* were selected as representative examples of bacteria, yeasts and fungi, respectively.

1. Introduction

Chitosan is a polysaccharide that is primarily composed of D-glucosamine repeating units and shows some biological activities, such as immunological activity [1]. Chitosan and its derivatives have received increasing amounts of attention as useful, biomedical polysaccharides due to the biocompatibility, biodegradability, and non-toxic properties of these compounds [2–4]. Chitosan and its derivatives show antimicrobial and antifungal activity [5] because they inhibit the growth of a wide variety of bacteria and fungi [6,7]. Currently, these biopolymers are limited to food applications [8].

Chitosan and β-cyclodextrin-grafted chitosan with different molecular weights were evaluated as antimicrobial agents against different microorganisms [9]. Cyclodextrins (CDs) are cyclic oligosaccharides that are composed of monosaccharide units, which are covalently linked together by 1,4-glycosidic linkages to form torus-like structures (Fig. 1). There are three main types of cyclodextrins: α, β, and γ types of six-, seven- and eight-member macro-rings of cyclic maltose [10,11]. Due to its hydrophobic cavity [10,12], grafting of CD onto chitosan can result in the formation of molecular carriers that possess cumulative inclusion effects. The transport properties and size specificity of CDs, as well as the controlled release ability of the polymeric matrix including CD-grafted chitosan derivatives and their mechanisms of host–guest complex formation with organic molecules, have been reviewed [10].

Chitosan grafts with biopolymers are used in many applications, such as in food, wastewater treatment, chromatographic support, enzyme immobilization and cosmetics. They are also used in biomedical and pharmaceutical applications, such as in drug and gene delivery, tissue engineering, wound healing and antimicrobial, antiviral and immune-adjuvant strategies [7]. Such grafts have been used in the form of structures, artificial skin, and sustained release materials for drugs, as well as in various industrial fields [8]. The properties of chitosan hydrogels prepared by irradiation depend on the preparation conditions, including the feed solution composition and the concentration and irradiation dose used to achieve the highest gelation degree. The prepared hydrogel possesses good pH-sensitivity and obeys Fickian diffusion behavior at pH = 1 and non-Fickian diffusion behavior at pH = 7. This finding indicates that this hydrogel would act as a good carrier for colon-specific drug delivery systems. *In vitro* drug release experiments have showed a promising ability for the prepared hydrogels to not only control the release site but also the release rate [1].

Recently, there has been a growing interest in chemical modification of chitin and chitosan to improve the solubility of these

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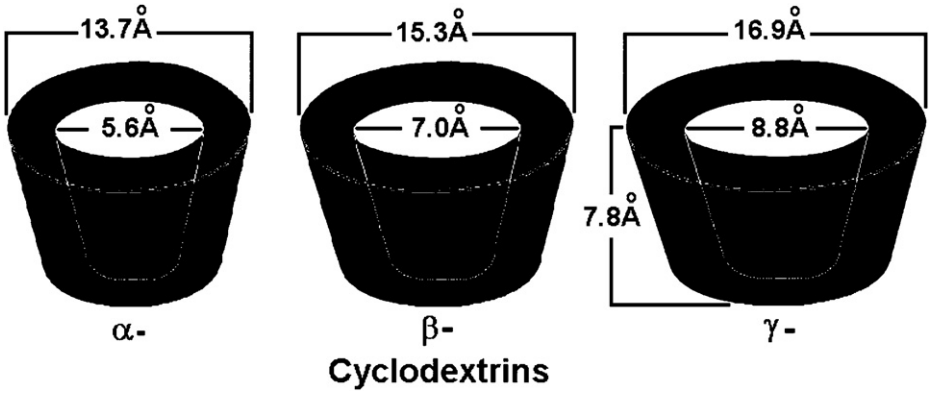

Fig. 1. Chemical structure of CDs (dimensions in Å).

compounds and widen their industrial applications [13]. Chitosan has been modified with 5-fluorouracil to serve as an *in vitro* antitumor drug delivery system [14]. Chitosan has also been modified with different simple organic and polymeric materials, as well as with multi-walled carbon nano-tubes (CNTs), to be used in ion-exchange, for example [15–17]. All the above-mentioned characteristics prompted us to study the modification of chitosan into the desired products involved in this work.

2. Experimental

2.1. Materials and characterization techniques

All experiments were conducted at the laboratories of the Faculty of Sciences at King Abdulaziz University. All chemicals were purchased from Aldrich, Milwaukee, WI, USA and were used as received unless otherwise mentioned. FT-IR spectroscopic analysis was performed using a JASCO model FT-IR 310 spectrophotometer. Thermal properties were investigated using a Shimadzu Thermal Analyzer with a scan rate 10.0 °C/min in an air atmosphere. Scanning electron microscope (SEM) images were taken using a JEOL JSM 6360 LV electron microscope at King Abdulaziz University, KSA. ICP-AES spectrophotometry was conducted using an Optima 8X00 ICP-OES Spectrometer, PerkinElmer, to determine the concentration of metal ions at wavelengths of 220.353 nm and 324.754 nm for Pb^{+2} and Cu^{+2} ions [18], respectively.

2.2. Blending of chitosan with carbohydrates

Approximately 1.0 g of chitosan (**CS**) was dissolved in 50 ml of 2 wt% aqueous acetic acid solution. Then, 1.0 g of the designated carbohydrate was added under agitation for 12 h at room temperature, and the product was air dried on a glass plate to obtain the products (**P1**, **P2**, and **P3** corresponding to glucose (**1**), lactose (**2**) and β-cyclodextrin (**3**), respectively). This procedure was repeated, and after the agitation step, approximately 0.2 ml of glutaraldehyde (**GA**) was added to achieve crosslinking of the modified **CS**. The reaction mixture was agitated for a further 20 min and finally air dried on a glass plate. The obtained crosslinked **CS** products were designated as **P1a**, **P2a** and **P3a**.

2.3. Blending of CS with cellulose triacetate (*4*)

First, 0.5 g cellulose triacetate (CTA) (**4**) was dissolved in 50 ml $CHCl_3$ and stirred for 6 h. Approximately 0.5 g of **CS** was dissolved in a mixture of 40 ml acetic acid and 50 ml $CHCl_3$ by stirring for 6 h and was then added to the solution of **4** while stirring over a period of 10–15 min. After additional stirring for 12 h, a few drops of water were added, and the mixture was then neutralized with NaOH solution. The precipitated product (**P4**) was filtered, washed thoroughly with chloroform and finally dried at 40 °C under reduced pressure for 24 h.

2.4. Modification of CS with phthalic anhydride (*5*)

The phthalic anhydride modification procedure was similar to a previously published procedure [19], with a slight modification. A suspension of 0.5 g of **CS** in 15 ml DMF was prepared under agitation and mixed with a solution of 1.4 g of phthalic anhydride (**5**) in 15 ml of DMF. The mixture was heated at 130 °C in an oil bath under continuous stirring in a nitrogen atmosphere for 5–7 h. The product (**P5**) was then filtered, washed thoroughly with ethanol and distilled water and dried at 40 °C under reduced pressure for 24 h.

2.5. Modification of CS with glycidyltrimethylammonium chloride (*6*)

Approximately 0.4 ml of glycidyltrimethylammonium chloride (GTMAC) (**6**) was added to a suspension of 0.5 g of **CS** in 50 ml of water with continuous stirring for 24 h at 80 °C. Ethanol was carefully added to the mixture, followed by acetone. The suspended beads turned from translucent or semitransparent into small white beads. The product (**P6**) was then separated by filtration and dried at 60 °C under reduced pressure for 24 h. After drying, 1 ml of glutaraldehyde (**GT**) was added as a crosslinking agent to produce a crosslinked, **CS**-supported quaternary ammonium salt (**P6a**).

2.6. Modification of CS into succinic acid derivatives

The modification with succinic acid derivatives was similar to a previously published procedure [16] with a slight modification. Approximately 1 g of succinic acid (**7**) was added to 0.5 g of **CS** suspended in 30 ml of DMSO and stirred for 24 h at 60 °C. The suspension was maintained at a pH of 10–12 with a 15% aqueous solution of sodium hydroxide. Succinic acid-modified **CS** (**P7**) was precipitated and dried under reduced pressure at 40 °C for 24 h. This procedure was repeated with dimercaptosuccinic acid (**8**) instead of **7**, which led to the formation of dimercaptosuccinic acid-modified **CS** (**P8**).

2.7. Treatment of CS derivatives with metal ions

Standard solutions of the investigated metals as nitrate salts were prepared by dissolving 1 g of the metal nitrate in 100 ml of distilled water. Then, 50 ml of the metal nitrate solution was added