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A new synthetic methodology for the preparation of biocompatible and organo-soluble barbituric- and thiobarbituric acid based chitosan derivatives for biomedical applications

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ABSTRACT

Chitosan's poor solubility especially in organic solvents limits its use with other organo-soluble polymers; however such combinations are highly required to tailor their properties for specific biomedical applications. This paper describes the development of a new synthetic methodology for the synthesis of organo-soluble chitosan derivatives. These derivatives were synthesized from chitosan (CS), triethyl orthoformate and barbituric or thiobarbituric acid in the presence of 2-butannol. The chemical interactions and new functional motifs in the synthesized CS derivatives were evaluated by FTIR, DSC/TGA, UV/VIS, XRD and ^{1}H NMR spectroscopy. A cytotoxicity investigation for these materials was performed by cell culture method using VERO cell line and all the synthesized derivatives were found to be non-toxic. The solubility analysis showed that these derivatives were readily soluble in organic solvents including DMSO and DMF. Their potential to use with organo-soluble commercially available polymers was exploited by electrospinning; the synthesized derivatives in combination with polycaprolactone delivered nanofibrous membranes.

1. Introduction

Chitosan (CS) is a natural biopolymer consisting of β(1.4) 2-amino-2-deoxy-D-glucopyranose (GlcN) repeating units in addition a small number of N-acetyl-D-glucosamine (GlcNAc) residues [1,2]. CS has numerous applications due to its excellent biocompatibility, biodegradability, nontoxicity, adsorptive properties, film-forming ability, and antimicrobial activity [3]. However, the insolubility of CS in water and most of the organic solvents limits its applications in various advanced biomedical fields. Therefore, to impart better biological activity and improved physical properties like solubility in organic solvents, special attention has been paid to its chemical modifications or derivatizations [2]. For this purpose, the presence of reactive primary amine and hydroxyl groups on the CS backbone provides a possibility for chemical modifications to prepare new materials with desired chemical, physical and biological properties [4].

Chitosan derivatives in particular organic solvents soluble derivatives have huge demand, because such materials could be used with a variety of organic solvents soluble synthetic polymers. Such combinations have useful properties such a degradation time can be tuned according to the required applications. CS chemical modifications were able to exhibit better biochemical, physiochemical and biological properties such as improved solubility [5,6], controlled drug release [7], wound healing properties [8], cell adhesion [9,10], antibacterial applications [11], antioxidant properties [12], better carrier efficiency for gene therapy [13].

Thus chemical modifications of CS are highly desired to obtain derivatives with the improved properties [2]. The existing methods of CS chemical modification include quarternization [14,15], acylation [16], alkylation [17,18], carboxyalkylation [19,20], hydroxyalkylation [21], mesylation [22], sulphation [23], thiolation [24], benzoylation [25] and graft copolymerization [26].

N-Reductive benzylation type reaction has been reported for the synthesis of organo-soluble materials, however this procedure lost its potential due to the non-availability of the starting materials [27]. Later, Morimoto et al. reported the synthesis of organo-soluble chitosan derivatives which were obtained by introducing phenol side chains into the amino group of the chitosan [28]. An effective procedure should utilize commercially available cheap and frequently available starting materials.

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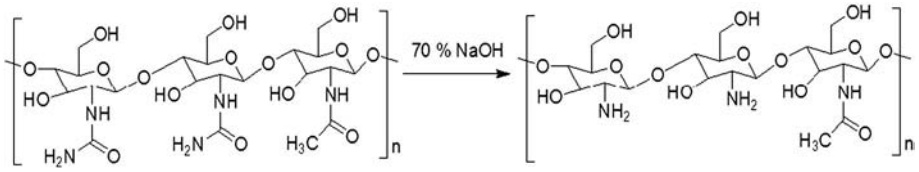


Scheme 1. Synthetic representation of chitosan from chitin.

In this paper we report a new method for the synthesis of novel organo soluble CS derivatives. In this new methodology we have found that commercially available barbichuric- and thiobarbichuric acid on reaction with chitosan in the presence of triethylorthoformate afforded the organo-soluble compounds under simple reaction conditions. The chemical and physical properties were studies and their solubility in various organic solvents was investigated and their further potential in the fabrication of electrospun nanofibrous sheets in the presence of organic solvents was studied.

2. Experimental

2.1. Materials

Chitosan (degree of deacetylation (DD) 78–98%, intrinsic viscosity 30.78 mL/g, Mw: 110019.06 g/mol) was synthesized in our laboratories (Scheme 1). Hydrochloric acid (HCl), barbituric acid and thiobarbituric acid were purchased from Merck (Germany). Triethyl orthoformate (98%) was purchased from Alfa Aesar. NaOH was purchased from Sigma Aldrich. DMSO and DMF were purchased from Lab-Scan Analytical Sciences, (Thailand).

2.2. Synthesis of CS derivatives (CSDB and CSDT)

Barbituric acid or thiobarbituric acid (0.5 g) was dissolved into 10 mL of 2-butanol and stirred the solution for 30 min at 80 °C. Once the solution was clear, respective amounts of triethyl orthoformate and chitosan were added and the solution was heated and stirred at 80 °C for 4 h. The obtained products were filtered in the hot state and were washed with the ethanol and dried at room temperature. The (CSDB) and (CSDT) codes were used to represent barbituric acid and thiobarbituric acid based CS derivatives, respectively (Scheme 2).

^{1}H NMR spectra were recorded in DMSO-d_6 and CH_3COOH/CF_3COOH with Bruker AM 500 spectrometer (Rheinstetten–Forchheim, Germany) operating at 400 MHz. ^{1}H chemical shifts are reported in δ (ppm). Splitting patterns were as follows s (singlet), d (doublet), t (triplet), and m (multiplet).

2.2.1. ^{1}H NMR analysis of CSDB (Fig. 1a)

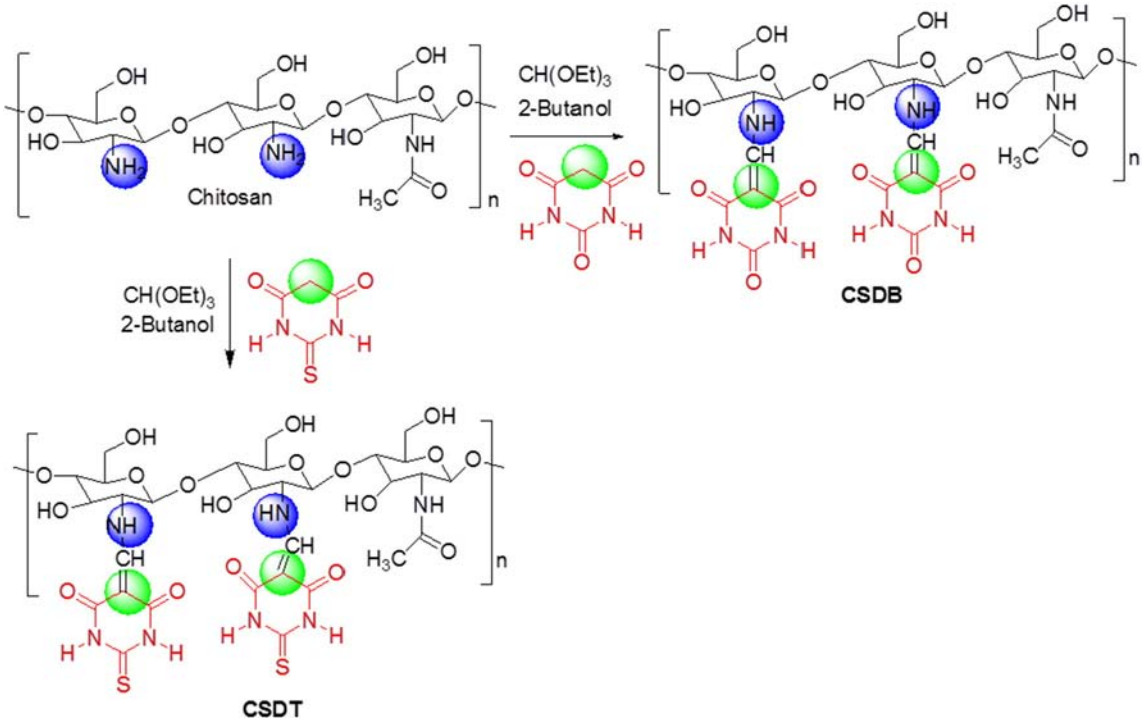
^{1}H NMR (d_6-DMSO, δ, ppm); 1.9 (s, COC***H*****$_3$** acetamide), 3.2–3.4 (m, CS ring), 8.66 (s, =C***H***NH), 9.95 (s, CON***H***CO).

2.2.2. ^{1}H NMR analysis of CSDT (Fig. 1b)

^{1}H NMR (D_2O/1% CF_3COOD, δ, ppm); 1.73 (s, COC***H*****$_3$** acetamide), 3.36–3.55 (m, CS ring), 7.87 (s, =C***H***NH), 8.12 (s, CON***H***CS).

2.3. FTIR analysis

The infrared (FT-IR) spectra were recorded on photo acoustic mode at the frequency range of 4000–400 cm^{-1} with 256 consecutive scans at 8 cm^{-1} resolution on a Thermo-Nicolet 6700 P FTIR Spectrometer (USA).



Scheme 2. Schematic representation of synthesis of CSDB and CSDT novel derivatives from CS.