Communications to the Editor

Synthesis of *N*-[(3′-Hydroxy-2′,3′-dicarboxy)-ethyl]chitosan: A New, Water-Soluble Chitosan Derivative†

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Chitin, which is (1,4)-linked poly(2-acetamido-2-deoxy-β-D-glucose), often referred to as poly(*N*-acetyl-D-glucosamine), is one of the most abundant natural polysaccharides available which contains nitrogen.[1] It is primarily obtained as a byproduct of the seafood industry, where it is isolated from the decalcified shells of various crustaceans. It can also be found in most insects and various fungi. Because of the highly crystalline nature of chitin, it is not readily water-soluble. Since this lack of water solubility limits the practical usefulness, a considerable amount of research has been conducted in methods to render chitin water-soluble. One of the earliest attempts was alkaline deacetylation of the *N*-acetyl groups to afford the free primary amines.[2] Such (1,4)-linked poly(2-amino-2-deoxy-β-D-glucose), also referred to as poly(D-glucosamine), is commonly called chitosan.

Although chitosan is not inherently water-soluble, it can be made to dissolve under various conditions. Examples are dissolution in aqueous acidic medium,[3] deacetylations ranging from 45 to 55%,[4] changes in polymer morphology and molecular weight,[5] and formation of microcrystalline chitosan.[6] Chitosan can also be rendered water-soluble by covalent chemical reactions, including alkoxylation,[7] carboxymethylation,[8] acylation,[9] and graft polymerization.[10] All of these chemical changes can render the polysaccharide water-soluble depending on the level of molar substitution (MS) of the chemical entity onto the polymer. In some cases, for example, carboxymethylation, the chitosan is made amphoteric by nature of the substitution. Amphoteric chitosan is particularly interesting since manipulation of the MS and solution pH can have pronounced effects on the polymer's solution behavior.

We wish to report a novel approach to forming a new type of amphoteric chitosan derivative made by the reaction of chitosan with *cis*-epoxysuccinic acid (*cis*-ESA, TCI America) (**2**, Scheme 1).[11]

The reaction occurs under alkaline conditions where the chitosan, previously decrystallized by reaction with a carboxylic acid, like lactic acid, initially swells but does not dissolve. Addition of **2** results in covalent derivatization of the chitosan to afford **3**. Progress of the reaction could be followed by visual inspection. The initially heterogeneous reaction mixture shows a gradual clearing and thickening as the polymer reacts with the oxirane and slowly dissolves into the alkaline reaction medium. We were also able to follow the course of the reaction by mid-infrared spectroscopy. The FT-IR spectra showed a gradual disappearance of the *cis*-ESA characteristic bands (Figure 1).

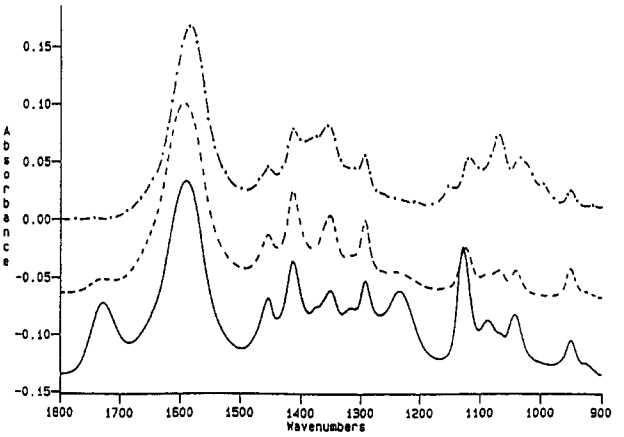
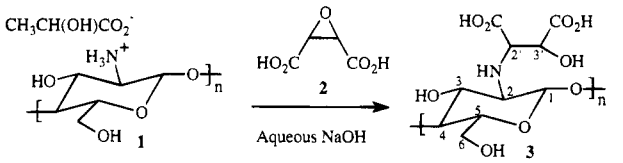


Figure 1. FT-IR spectroscopy to study the starting epoxide and reaction mixtures taken at 0 (—), 24 (---), and 48 (-·-) h.

Scheme 1. Reaction Conditions for Chitosan (as the Chitosan Lactate Salt) with *cis*-Epoxysuccinic Acid



The FT-IR spectrum of the neat *cis*-ESA is characterized by two intense bands, one in the carbonyl stretching region at 1724 cm^{-1} and the other in the epoxide C–O stretching region at 1235 cm^{-1}. The spectra showed a gradual disappearance of both of these bands as the reaction proceeded. Within 48 h, they disappeared completely. The band at about 1600 cm^{-1}, due to dicarboxylic acid carbonyl stretching modes, remained intense. The C–O stretching region shows some interesting changes. At the start of the reaction, the three prominent bands are observed at 1130, 1088, and 1043 cm^{-1}. Of these, the first one was the most intense. As the reaction proceeds, one observes rearrangement of relative band intensities: 48 h after the reaction, the peak positions are at 1120, 1070, and 1035 cm^{-1}. The most intense band in this case is the middle one. We feel this is due to alkyloxylation of chitosan by *cis*-ESA. Assignments of these other main bands will be included in a more detailed publication.

^{1}H and ^{13}C NMR characterization of the final product demonstrated that the new product is actually a random terpolymer containing three distinct moieties: (I) *N*-(3′-hydroxy-2′,3′-dicarboxy)ethyl-β-D-glucosamine, (II) β-D-glucosamine, and (III) *N*-acetyl-β-D-glucosamine. Initial assignments were made in aqueous 17% CF_3CO_2D solutions at 55 °C, which had a deleterious effect on the

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† Numbering corresponds to that given in **3** in Scheme 1.
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Table 1. Assignment of ^1H and ^{13}C Chemical Shifts of the Reaction Product of *cis*-Epoxysuccinic Acid and Chitosan in 17% CF_3CO_2D at 55 °C

structure[a]	carbon location	proton[b]	carbon[b]
I	1	5.02	98.0
I	2	3.37	63.6
I	3	4.08	71.0
I	4	3.98	77.8
I	5	3.70	75.6
I	6	3.70, 3.90	61.1
I	2′	4.85	63.5, 63.6
I	3′	4.85	69.3, 69.5
II	1	4.86	98.6
II	2	3.18	56.9
II	3	3.90	71.7
III	1	4.58	102.2
III	2	3.79	56.7
III	3	3.62	79.8
III-CH_3	CH_3	2.04	23.0

[a] (I) *N*-(3′-Hydroxy-2′,3′-dicarboxy)ethyl-β-D-glucosamine. (II) β-D-Glucosamine. (III) *N*-Acetyl-β-D-glucosamine. [b] In ppm from external TMS using 17 wt % CF_3COOD at 55 °C.

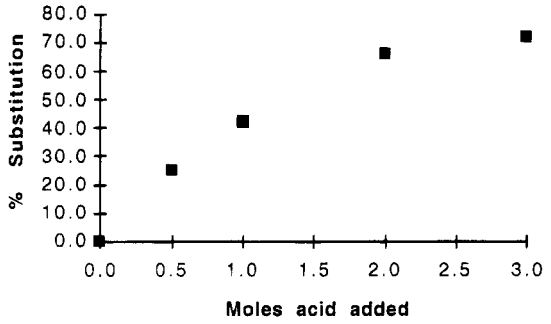

Figure 2. Percentage of nitrogen substitution versus molar treatment level of *cis*-epoxysuccinic acid run for 24 h at 85 °C.

polysaccharide backbone but did not change the nitrogen substitution levels. Later analyses were run in D_2O, and chemical shifts were determined by comparisons with acid-dissolved materials. The acid-dissolved polymers were analyzed by heteronuclear and homonuclear chemical shift correlation maps. For the purpose of this communication, the chemical shift data for assigned ^1H and ^{13}C signals are shown in Table 1.

Several examples of the product were synthesized by varying the molar amount of *cis*-ESA added per mole of glucosamine monomer available (Figure 2). The starting chitosan lactate used in these reactions is a commercial material obtained from crab shells (Kytamer L, Amerchol Corp.) known to have a weight-average molecular weight of approximately 350 000–700 000. The chitosan is approximately 85% deacetylated as determined by NMR. Each reaction was run for 24 h at 85 °C, and the subsequent substitution levels were determined by NMR.

At lower epoxide treatment levels, the efficiency of the reaction is high as a large number of available reactive amine sites exists. As the treatment level increases, more reagent is available for reaction, but available reactive amine sites are rapidly diminished, and the efficiency of the reaction drops to the point where addition of 3 mol of reagent becomes nearly redundant.

The new derivative is water-soluble over a broad pH range, particularly on the alkaline side. Aqueous solubility is influenced by molecular weight and reagent substitution; lower molecular weight chitosan and higher substitution levels both help to improve solubility. At pH below 5.0, the product has been observed to precipitate. Precipitation is particularly evident when higher molecular weight chitosan and higher substitution levels are employed. Apparently, cationization of the D-glucosamine nitrogens in the acidic medium does not provide the polymer with sufficient hydrophilicity to overcome the lipophilic nature of the protonated dicarboxylic acid moiety. Such behavior has been observed for other amphoteric chitosan derivatives.[12]

In attempting to ascertain the molar substitution level of the oxirane onto chitosan at a 1.5 molar treatment level, we were hampered by high viscosity in the aqueous NMR solutions. A molar treatment of 1.5 should yield a product with approximately 50% of the available nitrogens derivatized. It appears that at this level of substitution, a synergistic, ionic cross-linking may be occurring, thus leading to the observed high viscosity. This hypothesis is supported by aqueous FT-IR examination of these viscous solutions which shows, at neutral pH, an unusually strong stretching band for NH_2^+. We are investigating this anomaly further.

Of particular interest in the NMR analysis of the reaction product was the apparent absence of substitution of the oxirane onto the hydroxyl groups of the chitosan under the reaction conditions described. Figure 2 most clearly demonstrates that the epoxide reacts only on the available primary nitrogens as the substitution levels off at approximately 75% regardless of the amount of *cis*-ESA employed. This data is supported by combustion analysis, which indicates a comparable decrease in the molar percentage of nitrogen. The reaction appears to be quite selective, preferring the more nucleophilic glucosamine nitrogen. To verify this selectivity, we attempted to react the oxirane with water-soluble cellulose (Polymer JR, Amerchol Corp.) under comparable conditions. Cellulose is (1,4)-linked poly(2-deoxy-β-D-glucose), which is structurally similar to chitosan but lacks an amino group at the 2-carbon position. These reactions failed to afford a derivatized product.

Chitosan continues to generate interest as a useful biopolymer in multiple applications. It has been demonstrated that chitosan can now be rendered water-soluble by reaction with *cis*-ESA, affording a new, potentially useful derivative which should extend the versatility of this unique material.

References and Notes

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(10) For example, see: Singh, D. K.; Alok, R. R. *J. Appl. Polym. Sci.* **1994**, *53*, 1115.
(11) A typical procedure is as follows: In a 1000 mL round-bottom flask equipped with a strong overhead stirrer and a condenser was charged 400 g of a 5 wt % aqueous NaOH solution. To this was added 25.0 g of chitosan lactate (Kytamer L, Amerchol Corp., Edison, NJ) as a dry powder,and the slurry was vigorously stirred at room temperature for 1 h. To the swollen, heterogeneous mixture was charged 26.0 g of *cis*-epoxysuccinic acid, and the reaction mixture was heated to reflux for 36 h. The resulting homogeneous solution was cooled to 25 °C, and the pH was adjusted to 8.5 with 50% aqueous acetic acid. After filtration and dialysis, the aqueous residue was lyophilized to yield 24.3 g of the desired product as off-white flakes.
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