Preparation of Novel Biodegradable Polyampholyte: Partially Dicarboxylated Chitosan

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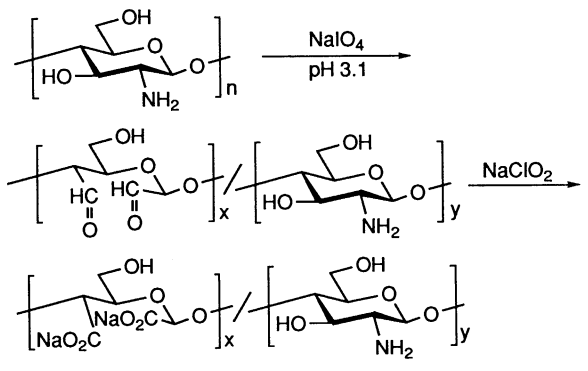
Amphoteric and biodegradable polycarboxylates having a varying amount of unreacted glucosamine moieties as the biologically active group as well as the biodegradable moiety were prepared by the partial oxidation of chitosan using sodium periodate at pH 3.0 followed by oxidation with sodium chlorite. Partially dicarboxylated chitosan containing more than about 50% unreacted glucosamine residue showed good biodegradation using activated sludge for 28 days.

Chitosan, the deacetylated derivative of chitin, is one of the abundant, renewable and biodegradable carbohydrate polymers, and many attempts have been made to develop new applications both in the industrial and medicinal fields. A high-molecular weight amphoteric polyelectrolyte having biodegradability is particularly needed both in the industrial and medicinal fields. The introduction of carboxyl groups into a polymer chain of chitosan will make it water-soluble as well as having an amphoteric nature. Carboxyl groups can be introduced into the chitosan by the carboxymethylation reaction and periodate oxidation. *O*- and *N*-Carboxymethylated chitosan has been prepared and evaluated for its biological activities and physicochemical behavior as a polyampholyte.[1] The partial ring-opening oxidation of chitosan by periodate will be a more preferable way to obtain the structurally well-defined carboxylated chitosan and the more highly carboxylated polyampholytes. However, dicarboxylation of chitosan via the ring-opening oxidation of periodate, so far, has not been established. Ohya *et al.* reported that the oxidation of chitosan by periodate produced degradation products. Only 6-*O*-glycolchitosan was oxidized by periodate without degradation to yield the dicarboxylated 6-*O*-glycolchitosan.[2] On the other hand, dicarboxylated polysaccharides, such as starch[3,4] and glucomannan[5] were reported to exhibit the calcium sequestration activities and biological activities. In this report, the preparation of partially dicarboxylated chitosan was studied, and its biodegradability was evaluated using activated sludge.

Partially dicarboxylated chitosan sodium salts (DC-Chs) having various degrees of dicarboxylation were prepared by partial conversion of the C2 and C3 moieties of the glucosamine units of chitosan into dicarboxylates via dialdehydes as shown in Scheme 1. The preparation of DC-Chs having a number-average molecular weight ($\overline{Mn}$) of 6190 and a dicarboxylation degree of 70 mol% [DC-Chs-6190(70)] is described as an example.



Scheme 1.

Table 1. Periodate oxidation conditions of chitosan and the yield, molecular weight and dicarboxylation degree of DC-Chs

	Periodate oxidation[a]			DC-Chs		
Entry	Conc. of HCl /mol dm^{-3}	pH[b]	Conc. of $NaIO_4$ /mol dm^{-3}	Isolated yield /%	$\overline{Mn}$ ($\overline{Mw}/\overline{Mn}$)	DC deg.[c] /%
1	0.03	3.1	0.005	24	4460(1.3)	40
2	0.05	2.0	0.008	26	4250(1.5)	48
3	0.03	3.1	0.008	28	5040(1.4)	51
4	0.025	4.1	0.008	22	4610(1.5)	49
5	0.02	5.2	0.008	12	3730(1.7)	48
6	0.05	2.0	0.015	31	5890(1.7)	73
7	0.03	3.1	0.015	34	6190(1.6)	70
8	0.025	4.1	0.015	25	6200(1.6)	69
9	0.02	5.2	0.015	11	4100(1.8)	75
10	0.03	3.1	0.031	33	6160(1.5)	82

[a] Chitosan (500 mg) was added to aqueous HCl (100 mL) at 0 °C and stirred for 15 min. $NaIO_4$ was then added and stirred at 0 °C for 2 h. [b] pH of the periodate reaction. [c] Dicarboxylation degree.

Chitosan (500 mg)[6] was added to a 0.03 mol dm^{-3} hydrochloric acid (100 mL) at 0 °C and stirred for 15 min. Sodium periodate (332 mg) was then added and stirred at 0 °C for 2 h in the dark (pH 3.1). After the reaction, the pH of the solution was adjusted to 7.6-7.8 with 1 mol dm^{-3} sodium hydroxide to precipitate diformylchitosan. The white precipitate was centrifuged (3500 rpm, 5 min) and washed thoroughly three times with chilled water to obtain the diformylchitosan. Thus, the obtained wet diformylchitosan was suspended in 30 mL water, nitrogen was bubbled into the solution at 0 °C for 30 min, and a solution of 5.4 g sodium chlorite in 10 mL water was added to the aqueous diformylchitosan. The pH of the solution was adjusted to 4.1 with acetic acid, and the mixture was stirred at 20 °C for 18 h. After the reaction, nitrogen was passed through the solution until a colorless solution was obtained. The pH of the solution was then raised to 10.5 with 1 mol dm^{-3} aqueous sodium hydroxide and concentrated to about 5 mL under vacuum. The solution was slowly poured into a large amount of ethanol (300 mL) with stirring to precipitate the polymer. The precipitated polymer was filtered and the polymer was washed with ethanol and dried under vacuum. The polymer was dissolved in 10 mL of water and dialyzed against distilled water for 3 d to remove any low-molecular weight fractions. The small

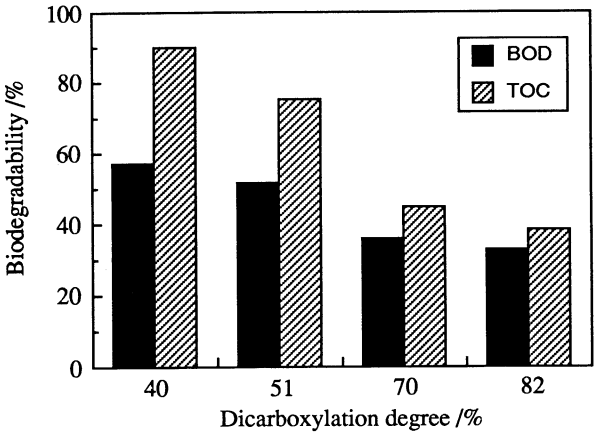


Figure 1. BOD- and TOC-Biodegradability of DC-Chs with activated sludge after 28 days.

amount of insoluble precipitate of unreacted chitosan was removed by centrifugation and filtered, and the filtrate was evaporated under vacuum before freeze-drying. Further drying was carried out under vacuum to obtain DC-Chs in 34% yield as a white powder.[7] The dicarboxylate content as determined by the trinitrobenzene sulfonic acid (TNBS) method[8] was 70 mol% ($\overline{M}n$ = 6190 and $\overline{M}w/\overline{M}n$ = 1.6 by GPC).[9]

Table 1 shows the periodate oxidation conditions as well as the yield, molecular weight and dicarboxylation degree of DC-Chs. The pH values were adjusted using hydrochloric acid. It was found that the C2 and C3 moieties of the glucosamine units of chitosan were oxidatively cleaved by sodium periodate to yield water-soluble dialdehydes in the slightly acidic solutions. In order to avoid cross-linking by the formation of intra- and intermolecular Schiff's base bonds, the periodate reaction was carried out in the pH range of 2 and 5. It was found that the molecular weight of the DC-Chs varies depending on the pH values of the periodate oxidation. The maximum yield and $\overline{M}n$ were obtained at a pH value of around 3. At pH values greater than 5, the yield of water-soluble diformylchitosan decreased significantly. The degree of oxidation increased with increasing sodium periodate concentration. It was also found that the yield of diformylchitosan was higher when hydrochloric acid was used instead of acetate buffer in the periodate reaction. Dialdehyde groups of diformylchitosan were further oxidized to the corresponding dicarboxylates by sodium chlorite at pH 4.1.

The biodegradability of DC-Chs containing unreacted sugar groups in the polymer chain was evaluated by measuring the biological oxygen demand (BOD) with a BOD Tester (Model 200F; TAITEC Corp., Koshigaya-shi) basically according to the OECD Guidelines for Testing of Chemical[4,10] at 25 °C for 28 d using an activated sludge freshly obtained from a municipal sewage treatment plant. Figure 1 shows the 28-d biodegradability as determined by the BOD values and total organic carbon (TOC) measurements. It was confirmed that DC-Chs containing more than about 50% unreacted sugar groups was regarded as readily biodegradable by the activated sludge. The biodegradability of DC-Chs was dependent on the content of the unreacted sugar moieties and similar tendencies were observed like that of dicarboxystarch.[4] The same biodegradation results were obtained from the TOC measurements before and after the biodegradation tests.

In conclusion, it was found that high-molecular weight DC-Chs was prepared by the periodate diformylation in slightly acidic solution at a pH value of about 3 with hydrochloric acid followed by subsequent dicarboxylation with sodium chlorite. The dicarboxylation degree increased with increasing periodate concentration. DC-Chs containing more than about 50 mol% unreacted sugar groups tended to readily biodegrade using the activated sludge.

References and Notes

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6 100% Deacetylated chitosan was purchased from Katokichi Co., Ltd. (Kannonji-shi).
7 The following spectral data for DC-Chs-6190(70) confirm the structure. IR (KBr) : 3418 (OH), 2941, 2884 (CH_2), 1616, 1396 (COONa), 1072, 1024 cm^{-1} (C-O-C). ^{13}C NMR (22.5 MHz : D_2O) : 61.7-63.1 (-$\underline{C}H_2$-), 72.1-75.0 [>$\underline{C}$H-CH(CH_2OH)-O-], 78.2 - 81.7 [>CH-$\underline{C}$H(CH_2OH)-O-, -$\underline{C}$H(OH)-], 100.9 - 102.5 (-O-$\underline{C}$H-O-), 174.9-177.1 (COONa).
8 A.F.S.A. Habeeb, *Anal. Biochem.*, **14**, 328 (1966).
9 The number-averge molecular weight *($\overline{M}n$)*, weight-averge molecular weight *($\overline{M}w$)* and molecular weight dispersion *($\overline{M}w/\overline{M}n$)* were measured by a gel permeation chromatography (GPC) using GPC columns (TSK gel G5000PW + G2500PW, TOSOH Co., Ltd., Tokyo) with a reflective index detector. 0.1 mol dm^{-3} phosphate buffer with 0.3 mol dm^{-3} NaCl at pH 6.8 was used as the eluent. The GPC system was calibrated with a poly(ethylene oxide) standard.
10 *OECD Guidelines for Testing of Chemicals, 301C, Modified MITI Test,* Organization for Economic Cooperation and Development, Paris.