Michael Reaction of Chitosan with Various Acryl Reagents in Water †

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Michael Reaction of Chitosan with Various Acryl Reagents in Water†

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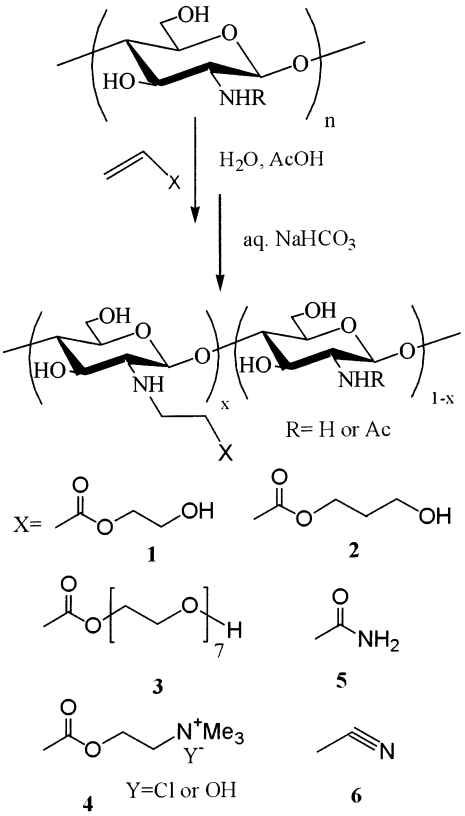
A Michael reaction of chitosan was conducted in water containing acetic acid with various acryl reagents. The degree of substitution could be controlled by temperature, reaction time, and the amount of acryl reagents. Although the modified chitosan derivatives with acrylic acid esters showed water-solubility, that with poly(ethylene glycol) acrylate, however, turned to water-insoluble material by lyophilization. Good biodegradation was observed in modified chitosan derivatives by standard activated sludge.

Introduction

Chitosan, a *N*-deacetylated chitin, is an attractive biomaterial owing to its effective biological properties such as gene delivary[2] and antibacterial activity.[3] Although chitosan is an interesting biomacromolecule, a weak point is that it is nonsoluble in water. The properity of water-solublility is important toward the further application of chitosan as biomaterials. To improve water-solubility, chemical modification of chitosan is necessary, and numerous works concerning this were introduced in a recent review.[4] The Michael reactions of chitosan have been developed as a new method for the chemical modification of chitosan[5] or partially deacetylated chitin,[6] and these resulting products were used as precursors for the construction of a chitosan−dendrimer hybrid.[7] Moreover, we have just reported on the Michael reaction of chitosan with acrylic acid.[1a] In this case, acrylic acid plays both the proton donor to make chitosan able to dissolve in aqueous medium and the reagent for the Michael reaction, so that water-soluble *N*-carboxyethylchitosan was successfully prepared in water. If water-soluble acryl reagents are applied for this reaction, novel types of functional groups will be introduced by a simple procedure. Herein, we report the Michael reaction of chitosan in water and AcOH (Scheme 1) with various acryl reagents such as AAm, AN, and some acrylic acid esters such as HEA, HPA, AETMAC, and PEG-A.

Scheme 1



Experimental Section

Materials. Chitosan SK10, 01P, D60, and D95 were supplied from Koyo Chemical Co., Japan. PEG-A, 80 wt % aqueous solution of AETMAC, and other reagents were purchased from Aldrich Co., Ltd.

General Methods. ^{1}H NMR spectra were recorded on JEOL A-500 NMR spectrometer. Proton chemical shifts (δ) are given relative to 3-(trimethylsilyl)propanesulfonate as an internal standard used in 0.5 M DCl in D_2O as solvent. For compound **3**, 0.1 M NaOD in D_2O was used as solvent, because of its insolubility in D_2O after lyophilization. The molecular weight was determined by means of GPC using

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pullulan as standards (column, Tosoh TSK Gel G4000pwxl and G3000pwxl; eluent, 0.5 M AcOH-0.5 M AcONa buffer; temp, 40 °C; flow rate, 1.0 mL/min; detector, RI). The solubility of the product in water was evaluated from the visual observation method where 50 mg of product was suspended in water (5 mL) for 1 day.

Biodegradation of Chitosan Derivatives. Biodegradation of chitosan and its derivatives with standard activated sludge was evaluated by BOD tester 100F (TAITEC Co., Japan) according to the previous report.[8] The typical procedure is as follows. A carbon-free inorganic buffer (1 L, pH 7.0) containing $MgSO_4$ (7.2 mg), $CaCl_2$ (27.7 mg), $(NH_4)_2SO_4$ (39.6 mg), $FeCl_3$ (0.6 mg), KH_2PO_4 (17 mg), K_2HPO_4 (43.5 mg), $Na_2HPO_4 \cdot 7H_2O$ (66.8 mg), and NH_4Cl (3.4 mg) was prepared. The supernatants of the standard activated sludge (20 mL), inorganic buffer (180 mL), and sample (100 mg) were placed in a fermentor at 25 °C. $Ca(OH)_2$ (1.0 g) was used as a trap of produced CO_2 gas. At the prescribed time, the amount of consumed O_2 gas was measured. The fermenteter, which did not include the sample, was used as a control. The net amount of consumed O_2 gas (mL) was evaluated as the difference of the sample and control. The theoretical amount of consumed O_2 (mL at 25 °C) means complete degradation of sample. Biodegradation (%) was calculated as follows:

$$\text{biodegradation (\%)} = [\text{experimentally consumed } O_2 \text{ (mL)}/\text{theoretical } O_2 \text{ (mL)}] \times 100$$

Preparation of Chitosan Derivatives. The typical reaction procedure is as follows: Chitosan (SK10, 1 g, NH_2=5 mmol) was dissolved in water (50 mL) containing AcOH (0.5 mL). Acryl reagent (2 equiv/NH_2) was added to the solution and stirred at 50 °C. After the prescribed time, $NaHCO_3$ powder was added to the reaction mixture to adjust the pH to 8−9, and the mixture was dialyzed against deionized water using a dialysis membrane (MW cut off 12 000) for 2 days. Finally, the dialysis product was lyophilized to obtain the chitosan derivative in excellent yield. DS was calculated from the peak area at δ 2.88−3.08 ppm of $-CH_{2b}-$ proton against 2.06 ppm of NHAc proton as follows except for compd **3**. DS = peak area (H) at δ 2.88−3.08 ppm/2

Data for **1** (DS = 0.44): ^{1}H NMR δ 2.06 (s, 0.45 H, NHAc), 2.88 and 2.95 (br, 0.88 H, $-CH_{2b}-CO_2-$), 3.20 (s, H-2 of GlcN), 3.30 (s, H-2 of *N*-alkylated GlcN), 3.6−4.1 (m, *N*-CH_{2a}- and $-CH_{2c}-$ of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.29 (s, 0.44 H, $-CH_{2d}-$OH), 4.87 (s, 0.41 H, H-1 of GlcN), 5.04 (br, 0.44 H, H-1 of *N*-alkylated GlcN).

Data for **2** (DS = 0.50): ^{1}H NMR δ 1.13−1.25 (m, 1.0 H, $-CH_{2d}-$), 2.07 (s, 0.45 H, NHAc), 2.91 and 2.97 (m, 1.0 H, $-CH_{2b}-CO_2-$), 3.20 (s, 0.35 H, H-2 of GlcN), 3.30 (s, 0.50 H, H-2 of *N*-alkylated GlcN), 3.6−4.1 (m, *N*-CH_{2a}- and $-CH_{2c}-$ of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.22 (m, $-CH_{2e}-$OH), 4.60 (br, 0.15 H, H-1 of GlcNAc), 4.87 (s, H-1 of GlcN), 5.04 (br, 0.50 H, H-1 of *N*-alkylated GlcN).

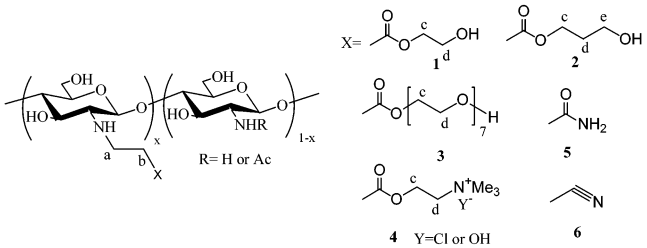
Data for **3** (DS = 0.39) in 0.1 M NaOD/D_2O: ^{1}H NMR δ 2.06 (s, 0.45 H, NHAc), 2.37 (br, 0.78 H, $-CH_{2b}-CO_2Na$), 2.53 (s, H-2 of GlcN), 2.70 (s, H-2 of *N*-alkylated GlcN), 3.4−4.1 (m, $N-CH_{2a}-$ and $-CH_{2c}-CH_{2d}-$ of PEG group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc). DS = peak area (H) at δ 2.37 ppm/2.

Data for **4** (DS = 0.43): ^{1}H NMR δ 2.06 (s, 0.45 H, NHAc), 2.92 (s, 0.86 H, $-CH_{2b}-CO_2-$), 3.19 (s, 3.87 H, NMe_3), 3.23 (s, H-2 of GlcN), 3.30 (s, 0.43 H, H-2 of *N*-alkylated GlcN), 3.6−4.1 (m, $N-CH_{2a}-$ and $-CH_{2c}-CH_{2d}-$ of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.60 (br, 0.15 H, H-1 of GlcNAc), 5.07 (br, 0.43 H, H-1 of *N*-alkylated GlcN).

Data for **5** (DS = 0.24): ^{1}H NMR δ 2.06 (s, 0.45 H, NHAc), 2.80 and 2.92 (br, 0.48 H, $-CH_{2b}-CONH_2$), 3.20 (s, 0.61 H, H-2 of GlcN), 3.30 (s, 0.24 H, H-2 of *N*-alkylated GlcN), 3.4−4.1 (m, $N-CH_{2a}-$ of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.61 (br, H-1 of GlcNAc), 4.88 (s, 0.61 H, H-1 of GlcN), 5.04 (s, 0.24 H, H-1 of *N*-alkylated GlcN).

Data for **6** (DS = 0.16): ^{1}H NMR δ 2.06 (s, 0.45 H, NHAc), 3.08 (s, 0.32 H, $-CH_{2b}-CO_2-$), 3.19 (s, 0.69 H, H-2 of GlcN), 3.30 (s, 0.16 H, H-2 of *N*-alkylated GlcN), 3.6−4.1 (m, $N-CH_{2a}-$ of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.60 (br, 0.15 H, H-1 of GlcNAc), 4.87 (br, H-1 of GlcN), 5.07 (br, 0.43 H, H-1 of *N*-alkylated GlcN).

Methylene signals ($-CH_{2a-e}$) of compounds **1**−**6** were assigned as the following formula



Results and Discussion

Figure 1 shows the ^{1}H NMR spectra of (A) original chitosan and (B) compound **1**. Typical signals at δ = 2.88−2.97 ppm assigned to methylene proton ($-CH_{2b}-COO-$) were observed. Furthermore, the H-1 proton signal attributed to the GlcN residue was shifted from δ = 4.89 to 5.04 ppm. The H-2 proton signal was also shifted from δ = ca. 3.20 to 3.30 ppm. These shifts were due to the substitution of the *N*-alkyl group to the amino groups of the GlcN residue. The signals attributed to the methylene proton ($-CH_{2b}$-COO-) were observed in compounds **2**−**6**. Additionally, the typical methylene ($-CH_{2d}-$) or methyl signals ($-NMe_3$) were also observed in compounds **2** or **4**, respectively (see experimental part). From these results, chemical modifications of chitosan shown in Scheme 1 were suggested.

Table 1 shows the results of the Michael reaction of chitosan (SK10) with various acryl reagents in aqueous AcOH medium. In some cases, the yield was excellent (85−96%). For acrylic acid esters such as HEA, HPA, PEG-A, and AETMAC, the DS of products (**1a**−**4a**) was remarkably higher than those for AAm **5a** or AN **6a**, which indicates esters were more reactive for the Michael reaction of chitosan

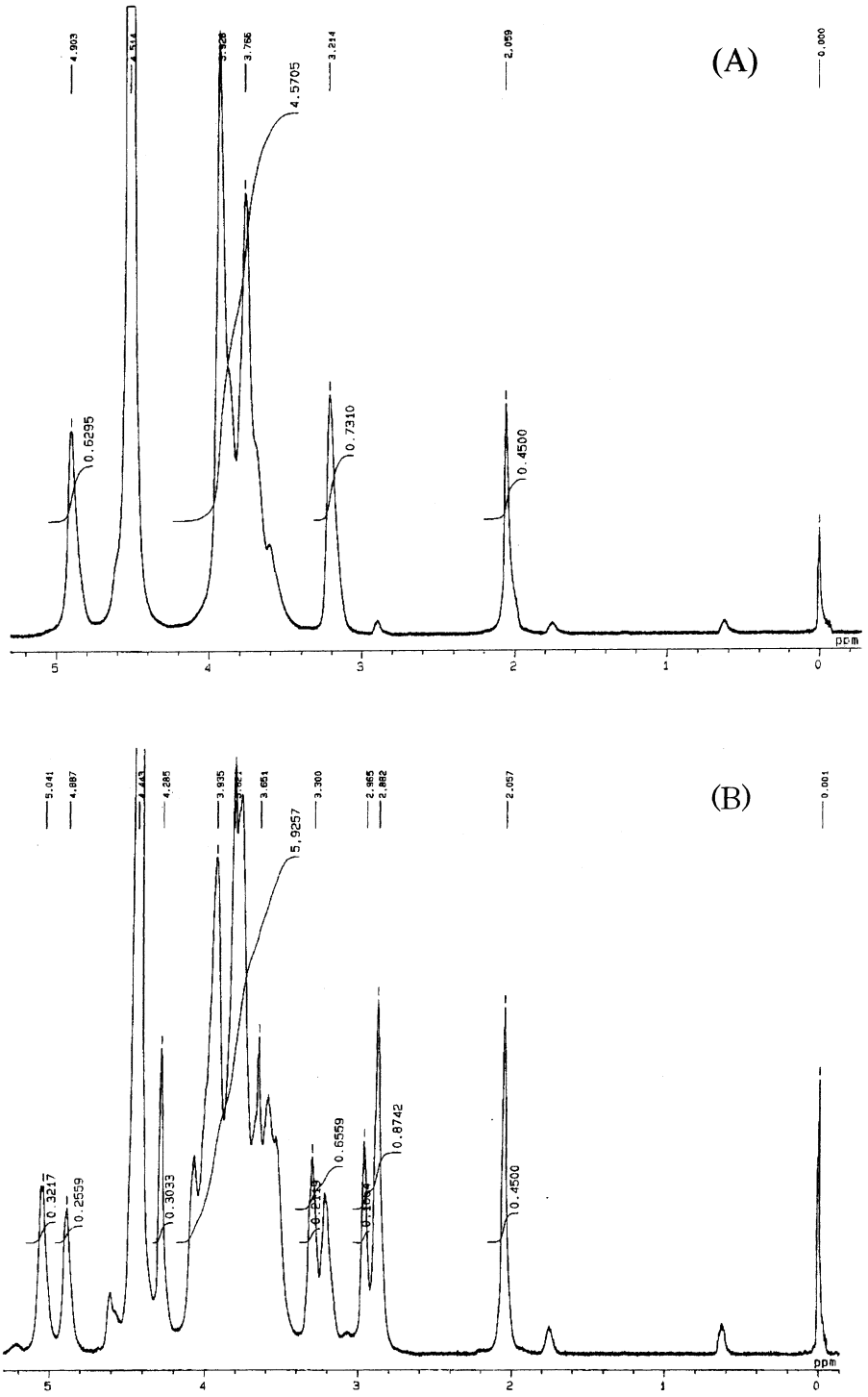


Figure 1. Typical ^{1}H NMR spectra of (A) chitosan (SK-10) and (B) compound **1**.

than for the latter. The molecular weight of products was almost at the same level compared with that of the original chitosan. Water-solubility was observed on the products having hydroxyl groups such as **1**, **2**, and **3** or quarter ammonium groups **4**. Although product **3** was soluble in water, it turned out to be insoluble after the lyophilizing process. The insolubilization of chitosan derivatives by the lyophilization process would be caused by the intermolecular hydrogen bond owing to PEG group, although its detailed mechanism was not clear at the present stage. The soluble pH range of water-soluble products was 1−11. The saponification of esters (**1** and **2**) was observed over pH 11, and the water-soluble *N*-carboxyethylchitosan sodium salt was formed. The structural novel point is that a variety of functional groups such as hydroxyl (**1**, **2**), PEG (**3**), quaternary ammonium (**4**), amide (**5**), and nitrile (**6**) could be introduced to chitosan with a quite simple procedure in aq. AcOH as medium. By the use of hydroxyethyl methacrylate, however, this reaction did not proceed under the condition in Table 1.

The effect of DDA and MW on the reaction of chitosan with HPA was summarized in Table 2. Almost similar DS

Table 1. Michael Reaction of Chitosan with Various Acryl Reagents[a]

compd	acryl reagent	product yield/%	product DS	MW (kDa) Mn	MW (kDa) Mw	solubility in H_2O
1a	HEA	85	0.44	37	95	yes
2a	HPA	92	0.50	24	73	yes
3a	PEG-A	87	0.39	27	53	yes[b]
4a	AETMAC	92	0.43	40	74	yes
5a	AAm	96	0.09	25	50	no
6a	AN	94	0.16	28	64	no

[a] Condition: acryl reagent = 2 equiv/NH_2, 50 °C, 2 days; chitosan, SK10 (Mn = 42 kDa, Mw = 80kDa). [b] Product was turned to insoluble after lyophilization.

Table 2. Effect of DDA and MW on the Michael Reaction of Chitosan with HPA[a]

chitosan code	chitosan DDA/%	MW (kDa) Mn	MW (kDa) Mw	product **2** DS
D60	60	73	130	0.27
SK10	85	42	80	0.50
01P	85	24	68	0.53
D95	95	110	390	0.47

[a] Condition: HPA = 2 equiv/NH_2, 50 °C, 2 days.

Table 3. Michael Reaction of Chitosan (SK10) under the Various Conditions

reagent	equiv	temp, °C	product time, day	product compd	product DS	solubility in H_2O
HPA	1	50	1	**2b**	0.18	no
	1	50	2	**2c**	0.22	yes
	1	50	3	**2d**	0.23	yes
	2	50	0.3	**2e**	0.14	no
	2	50	1	**2f**	0.26	yes
	2	70	1	**2g**	0.51	yes
	2	90	1	**2h**	0.55	yes
PEG-A	2	50	1	**3b**	0.29	yes
	4	50	1	**3c**	0.55	yes
AETMAC	1	50	1	**4b**	0.19	yes
	2	50	1	**4c**	0.34	yes
	4	50	1	**4d**	0.38	yes
	1	70	1	**4e**	0.48	yes
	2	50	4	**4f**	0.48	yes
AAm	10	50	2	**5b**	0.24	no
An	10	50	2	**6b**	0.49	no
	2	50	6	**6c**	0.18	no

of products were obtained for chitosan with DDA = 85−95%, whereas the DS was remarkably low in the case of DDA = 60%, which would be caused by a relatively higher concentration of reactive amino groups. The DS of products was slightly increased with a decreasing MW of chitosan with similar DDA (85−95%). All of the products in Table 2 were soluble in water.

Table 3 shows the results of the Michael reaction of chitosan (SK10) with some acryl reagents under the various conditions except for that in Table 1 (2 equiv 50 °C, 2 days). In the case of HPA, the DS values were increased with increasing (1) the amount of reagent, (2) the temperature, and (3) the reaction time. The lower DS of products under 0.18 did not dissolve in water. For PEG-A, the DS could be controlled from 0.29 to 0.55 by the amount of reagent. In the case of AETMAC, the product with DS = 0.19 dissolved in water, and the DS could be controlled from 0.19 to 0.48 by the reaction conditions. The products derived from AAm and AN, however, did not dissolved in water even at high DS (0.24 and 0.49), which was caused by the poor solubility of amide or nitrile in water.

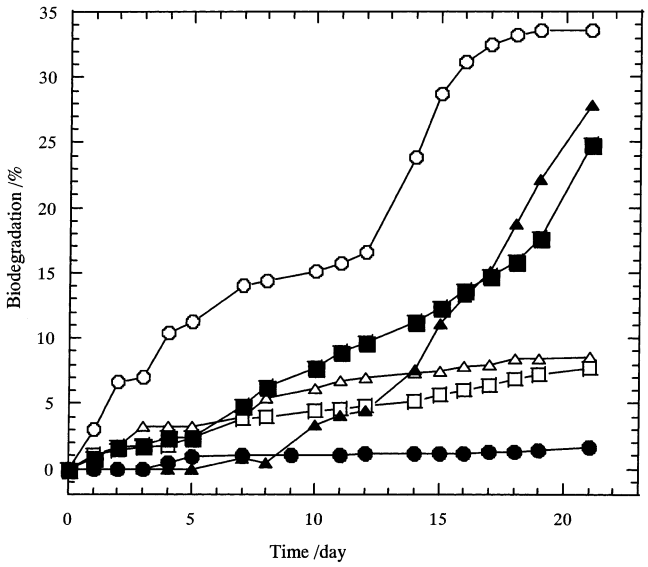


Figure 2. Time courses on the biodegradation of chitosan and its derivatives. The DS and solubility in water are listed in Table 4. ●, original chitosan; △, **1a**; ■, **3a**; ○, **4d**; ▲, **5b**; □, **6b**.

Table 4. Biodegradation of Chitosan and Its Derivatives[a]

sample	DS	solubility in H_2O	biodegradation, %
chitosan	0	no	1.6
1a	0.44	yes	8.6
2a	0.39	yes	5.0
3a	0.50	no[b]	24.8
4d	0.38	yes	33.6
5b	0.24	no	27.8
6b	0.49	no	7.7

[a] Sample, 100 mg; time, 21 days. [b] Water-insoluble product after lyophilization.

Figure 2 and Table 4 show the biodegradation of original chitosan and its derivatives by standard activated sludge. In any case, the biodegradability was enhanced by chemical modification compared with that of original chitosan. The highest biodegradability was shown in **4d** having a quaternary ammonium group. Compounds **3a** and **5b** modified with PEG and amide groups also showed good biodegradability. Moderate biodegradability was shown in **1a** and **6b** having hydroxyethyl and nitrile groups. These results suggest that biodegradation was much associated with the chemical structure of the chitosan derivatives. Thus, quaternary ammonium, amide, and PEG groups had the advantage for the biodegradation, but hydroxyethyl or nitrile group did not. On the other hand, the biodegradation was independent of the water solubility of chitosan derivatives. During the biodegradation test, further chemical modification did not occur. The mechanism for the biodegradation of chitosan derivatives is presumed as follows. First, glycoside or the ester linkage in the chitosan derivative is hydrolyzed by some enzymes such as glycosidase or lipase, etc., and then it is metabolized by some bacteria and microorganisms in standard activated sludge.

Conclusion

In this study, the chemical structure was defined by ^{1}H NMR spectra and *N*-alkylation of chitosan was confirmed. Water-soluble chitosan derivatives **1**−**4** were successfully obtained in aqueous AcOH medium. The chitosan derivatives having PEG, quaternary ammonium, and an amide group showed good biodegradation with standard activated sludge. This procedure will be useful for the novel chemical modification to prepare water-soluble and biodegradable chitosan derivatives.

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References and Notes

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(9) Abbreviations: AAm, acrylamide; AcOH, acetic acid; AcONa, sodium acetate; AETMAC, [2-(acryloyloxy)ethyl]trimethylammonium chloride; AN, acrylonitrile; DDA, degree of deacetylation; DS, degree of substitution; GlcN, D-glucosamine; GlcNAc, *N*-acetyl-D-glucosamine; GPC, gel permeation chromatography; HEA, hydroxyethyl acrylate; HPA, hydroxypropyl acrylate; MW, molecular weight; Mn, number average MW; Mw, weight average MW; PEG, poly(ethylene glycol); PEG-A, poly(ethylene glycol) acrylate; RI, refractive index.

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