Synthesis and properties of diethylaminoethyl chitosan

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A novel diethylaminoethyl chitosan was synthesized via Schiff-base intermediates. The C2 amino group in chitosan was protected from the reaction between benzaldehyde and chitosan to form *N*-benzylidene chitosan. After reaction with diethylaminoethyl chloride, the Schiff base was removed by reacting *O*-diethylaminoethyl-*N*-benzylidene chitosan and dilute ethanolic hydrochloride solution. The tensile strength of diethylaminoethyl chitosan was improved by incorporation of the diethylaminoethyl group in the C6 position of chitosan. Diethylaminoethyl chitosan showed pH-dependent swelling characteristics.

(Keywords: chitosan; diethylaminoethyl chitosan; *N*-benzylidene chitosan; synthesis; characterization)

INTRODUCTION

Several research groups have reported on the preparation of chitosan derivatives[1,2]. Attempts have been made to use chitosan for haemodialysis membranes[3], biocompatible materials[4], biodegradable sutures[5] and artificial skin substitutes[6]. Chitin, which is obtained mainly from the cuticle of a marine crustacean, has recently aroused great interest in its industrial and medical applications. Deacetylation of the acetamide group at the C2 position in the acetylglucosamine unit of chitin by alkaline hydrolysis yields chitosan, which is a cationic polyelectrolyte. Chitosan appears to be more useful than chitin, since it has both hydroxyl and amino groups that can be modified easily. Although many chitosan derivatives have been prepared, selective reaction of the diethylaminoethyl group on the C6 position of chitosan has not been reported yet.

We have already reported an insulin delivery system using a blend of poly(vinyl alcohol) and chitosan[7,8]. Amino groups on chitosan react with aldehyde to form a Schiff base, which can be easily removed after treatment with dilute ethanolic hydrochloride solution[9]. From knowledge of the swelling properties of chitosan in an acidic environment, the present study was conducted in an attempt to increase the pH sensitivity by deliberately incorporating amino groups in the glucosamine molecules. The present study aims to prepare the novel diethylaminoethyl chitosan (DEAE-chitosan) and to investigate its properties.

EXPERIMENTAL

Materials

Chitosan, whose degree of deacetylation was calculated to be 76% from the amino content, was purchased from Tokyo Kasei Co. (Japan), and was used after passage through a 200 mesh sieve. Diethylaminoethyl chloride hydrochloride (DEAE.HCl) was purchased from Aldrich Chemical Co. Inc. and was used after recrystallization from dioxane/ethanol (9/1 in volume). Benzaldehyde was purchased from Kokusan Chemical Co. (Japan).

Characterization

Infra-red spectra were measured on a Nicolet 5DX *FT*i.r. spectrophotometer. Wide-angle X-ray diffraction patterns were recorded with a flat-film camera using nickel-filtered Cu Kα radiation produced by a Rigaku (D/MAX, 111A) diffractometer. Mechanical properties were measured by an Instron-type universal testing machine (Tensilon/UTM-4-100, Japan), using samples of 5 mm width cut from the DEAE-chitosan film in the dry and wet states. The test was carried out using a crosshead speed of 4 mm min^{-1} and a gauge length of 20 mm. At least five samples of each specimen were tested. If disparity between the five readings exceeded $\pm 5\%$ of the mean value, testing continued until five consecutive readings within these limits were obtained. The average tensile strength and elongation at break of samples were calculated. Thermal properties of materials were measured using a differential scanning calorimeter (DuPont model 910) with a heating rate of 20°C min^{-1}. Thermogravimetric analysis (DuPont model 951) was used to investigate the thermal stability of DEAE-chitosan. Solid-state ^{13}C n.m.r. was conducted using IBM NR-100 machines. Proton and carbon frequencies were 100 MHz and 25 MHz, respectively.

Preparation of O-DEAE-chitosan

Figure 1 shows a schematic representation of the preparation of DEAE-chitosan. Powdered chitosan (3 g) was dissolved in 120 ml of 10 wt% acetic acid and diluted with methanol. Then 15.81 g of benzaldehyde was slowly dropped into the chitosan solution over 30 min to obtain *N*-benzylidene chitosan (B-chitosan), which was stored

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in an oven set at 60°C for 24 h to obtain a transparent gel. The gel was washed with methanol several times to remove any unreacted benzaldehyde, and freeze-dried for 24 h. Powdered B-chitosan (1 g) was swollen in pyridine/chloroform solvent at 60°C for 24 h and was reacted with 3.44 g of DEAE.HCl at 50°C for 24 h to prepare *O*-diethylaminoethyl-*N*-benzylidene chitosan (DEAE-B-chitosan). Powdered DEAE-B-chitosan (3 g) was suspended in 0.25 N hydrochloride ethanolic solution. The suspension was treated at room temperature for 24 h. The precipitates collected by a glass filter were washed several times with ethanol to eliminate any unreacted DEAE.HCl. The precipitates were washed again with acetone and ether, subsequently treated with 1 N NaOH solution and dried over P_2O_5 *in vacuo* at 110°C for 24 h to obtain DEAE-chitosan.

Determination of the degree of substitution

First, 50 mg of a sample was added to 0.3 N hydrochloride solution. The mixture was stirred to dissolve or to swell the sample. The average number of substitutions per pyranose unit was calculated by titrating potentiometrically with 0.1 N sodium hydroxide aqueous solution.

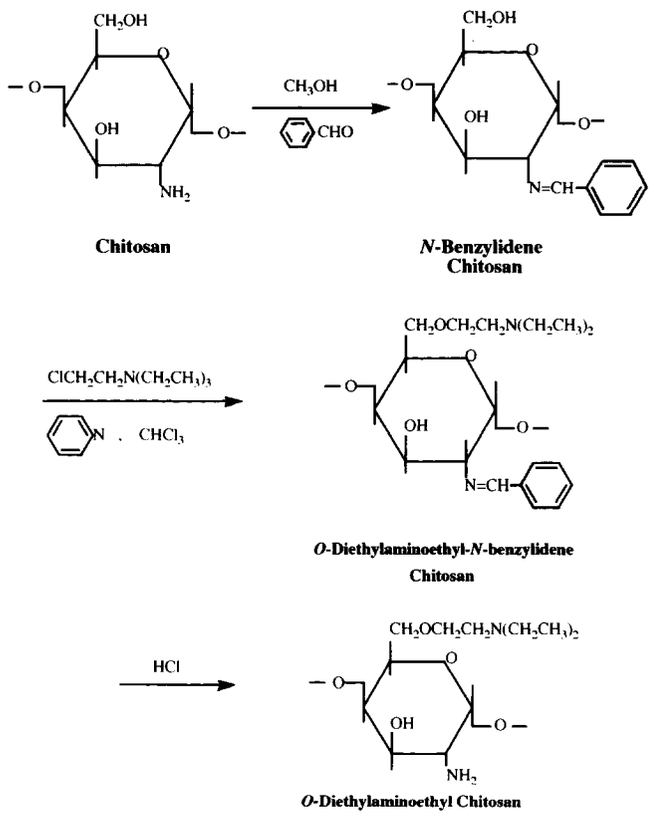

Figure 1 Reaction scheme for the synthesis of *O*-diethylaminoethyl chitosan

Preparation of O-*DEAE-chitosan membrane*

A given amount of DEAE-chitosan was dissolved in 100 ml of 5 wt% acetic acid solution to prepare the casting solution. The solution was filtered using a glass filter to remove dust. Glutaraldehyde was added as specified in *Table 1*. The samples were coded from G-1 to G-4. Before the solution became a gel, the casting solution was cast on a poly(methyl methacrylate) (PMMA) plate. The water was allowed to evaporate at 40°C in a convective oven for 24 h. After immersing the membrane in 1 N NaOH aqueous solution for 1 day, it was washed repeatedly with water to eliminate any unreacted glutaraldehyde. The membrane thickness was about 100 ± 10 μm.

Degree of swelling

The weight of a completely dried sample was measured directly, and the sample was dipped into a Petri dish filled with a different pH buffer solution at 37°C in an incubator. The degree of swelling of these samples was calculated with the following equation:

$$\text{degree of swelling } (Q_w) = (X_2 - X_1)/X_1 \qquad (1)$$

where X_1 and X_2 are the weights of dry and swollen samples measured at different times.

RESULTS AND DISCUSSION

Characterization

B-Chitosan was a transparent gel that did not melt upon heating at 200°C for 10 min and did not dissolve in organic solvents such as dimethylsulfoxide (DMSO), chloroform, formamide and dimethylformamide (DMF). DEAE-B-Chitosan was light brown in colour and did not dissolve in organic solvents such as DMSO, chloroform, formamide and DMF, but it was rather swollen in acetic acid solution. DEAE-Chitosan, on the other hand, dissolved easily in acetic acid solution.

Figure 2 showed the infra-red spectra of chitosan and chitosan derivatives. Although marked differences were not observed in the i.r. spectra between derivatives, characteristic peaks of aromatic C–H out-of-plane deformation appeared at 760 and 720 cm^{-1} for B-chitosan and DEAE-B-chitosan owing to the presence of benzaldehyde groups. Note that these characteristic peaks disappear in DEAE-chitosan, caused by treating

Table 1 Preparation and mechanical properties of DEAE-chitosan in dry and wet states

Sample	Amount of glutaraldehyde in casting solution (mol/g polymer)	Tensile strength (kg mm^{-2})		Elongation (%)	
		Dry	Wet	Dry	Wet
DEAE[a,b]	–	15.3	4.6	11.7	54
G-1	1.0×10^{-6}	16.2	5.2	12.1	58
G-2	2.5×10^{-6}	17.2	5.7	11.2	54
G-3	5.0×10^{-6}	18.9	6.4	10.4	43
G-4	1.0×10^{-5}	19.4	7.1	9.4	37
Chitosan	–	13.5	3.2	14.1	78

[a]Benzaldehyde = 8 mol/pyranose unit
[b]DEAE = 1.2 mol/hydroxyl unit of C6 position in chitosan

Figure 2 I.r. spectra of (a) chitosan, (b) *N*-benzylidene chitosan, (c) *O*-diethylaminoethyl-*N*-benzylidene chitosan, and (d) *O*-diethylaminoethyl chitosan

the DEAE-B-chitosan in hydrochloride ethanolic solution to remove the Schiff base. These Schiff bases used for *O*-diethylaminoethylation remained in the course of the reaction (from B-chitosan to DEAE-B-chitosan) and effectively protected the amino groups in the chitosan. Schiff bases were completely removed after treatment with hydrochloride ethanolic solution at room temperature. The intensity of the C–H stretching vibration of the methylene group in the region of 2850–2900 cm^{-1} increases for DEAE-B-chitosan and DEAE-chitosan. This confirms the introduction of the diethylaminoethyl group in the C6 position in the chitosan.

The degree of substitution (*DS*) of B-chitosan was 0.98. The *DS* value for the DEAE groups in the DEAE-B-chitosan and the DEAE-chitosan was 0.23%. This indicates that no release of DEAE groups in the DEAE-B-chitosan was found from the hydrochloride treatments.

Figure 3 shows the ^{13}C n.m.r. spectra of chitosan and chitosan derivatives. Similar to the i.r. analysis, a characteristic aromatic carbon appeared at 129 ppm for B-chitosan and DEAE-B-chitosan. Note that it is not seen in the spectra of chitosan and DEAE-chitosan. Changes were also noticeable for carbon in CH_3 and CH_2 groups at 23 and 59 ppm, respectively, owing to the presence of DEAE groups in DEAE-B-chitosan and DEAE-chitosan.

X-ray analysis

Figure 4 shows the wide-angle X-ray diffraction (WAXD) patterns of chitosan and chitosan derivatives. The WAXD pattern of chitosan shows the characteristic peak at $2\theta = 10°$ due to the presence of (0 0 1) and (1 0 0) and that at $2\theta = 20°$ caused by the presence of (1 0 1) and (0 0 2)[10]. For B-chitosan the peak at $2\theta = 10°$ disappeared, and the characteristic peak at $2\theta = 20°$ decreased. For DEAE-B-chitosan the intensity

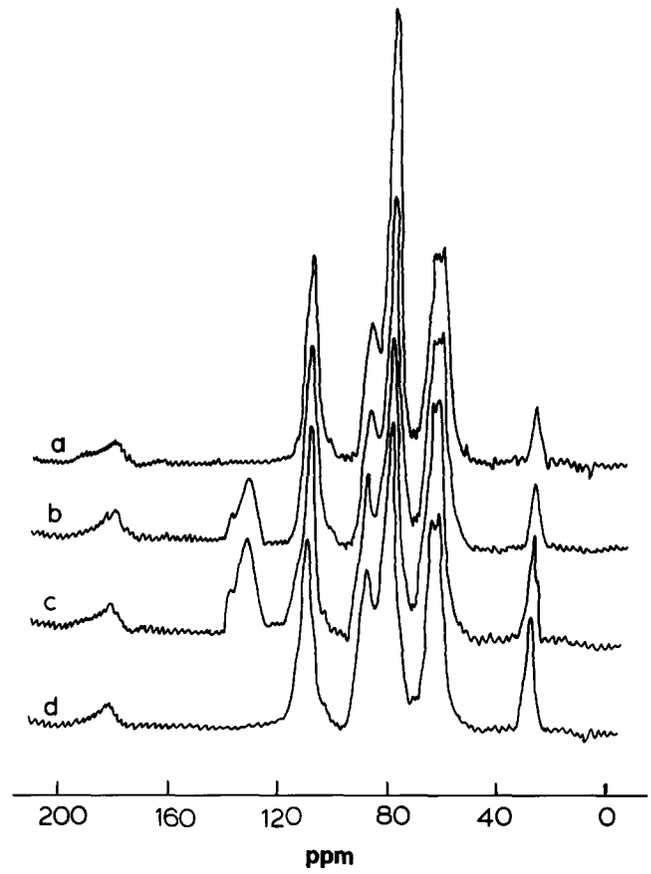

Figure 3 ^{13}C n.m.r. spectra for (a) chitosan, (b) *N*-benzylidene chitosan, (c) *O*-diethylaminoethyl-*N*-benzylidene chitosan, and (d) *O*-diethylaminoethyl chitosan

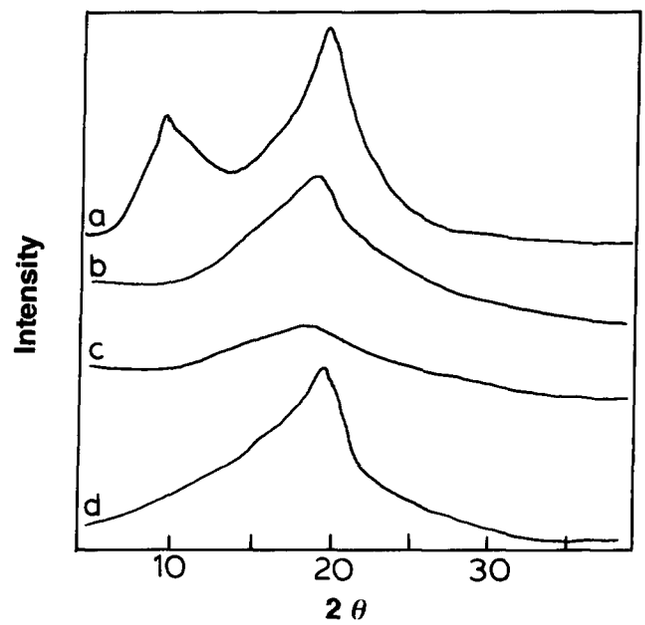

Figure 4 X-ray diffraction patterns of (a) chitosan, (b) *N*-benzylidene chitosan, (c) *O*-diethylaminoethyl-*N*-benzylidene chitosan, and (d) *O*-diethylaminoethyl chitosan

of the characteristic peak at $2\theta = 20°$ decreased more than that of B-chitosan. We thought that the decrease in crystallinity of chitosan derivatives was attributed to the deformation of the strong hydrogen bond in the chitosan backbone as the hydroxyl and amino groups were substituted by the benzylidene and diethylaminoethyl groups. Both derivatives gave a low crystallinity, indicating that they were considerably more amorphous than chitosan. Meanwhile, the characteristic peak of DEAE-chitosan at $2\theta = 20°$ increased in the X-ray diffraction pattern. It is believed that the regenerated amino groups formed the hydrogen bond again, resulting in the increase in the crystallinity.

Thermal properties

Figure 5 exhibits the d.s.c. thermograms of the chitosan derivatives. Chitosan showed an exothermic peak at around 330°C due to the degradation of the main chain[11]. B-Chitosan, DEAE-B-chitosan and DEAE-chitosan showed decreased exothermic peaks. In general, it is known that hydrogen bonds between polymer chains contribute to raising the degradation temperature, whereas the degradation temperature of the alkyl-substituted polymer may be decreased owing to loss of hydrogen bonding. Therefore, if the hydroxyls or amino groups were replaced by bulky groups such as benzylidene and diethylaminoethyl groups, hydrogen bonding in the polymer chains might be diminished, resulting in the smaller exothermic peak. Moreover, as the DEAE-chitosan recovers its amino group from the Schiff base, the intensity of the exothermic peak of DEAE-chitosan is greater than that of B-chitosan and DEAE-B-chitosan. These data coincide well with the X-ray analysis.

Figure 6 shows the thermogravimetric analysis (t.g.a.) of crosslinked DEAE-chitosan. The initial degradation temperature increased with the degree of crosslinking. That is, as the concentration of the crosslinking agent increased, the crosslinking density in the chain increased and thus the thermal stability could be enhanced.

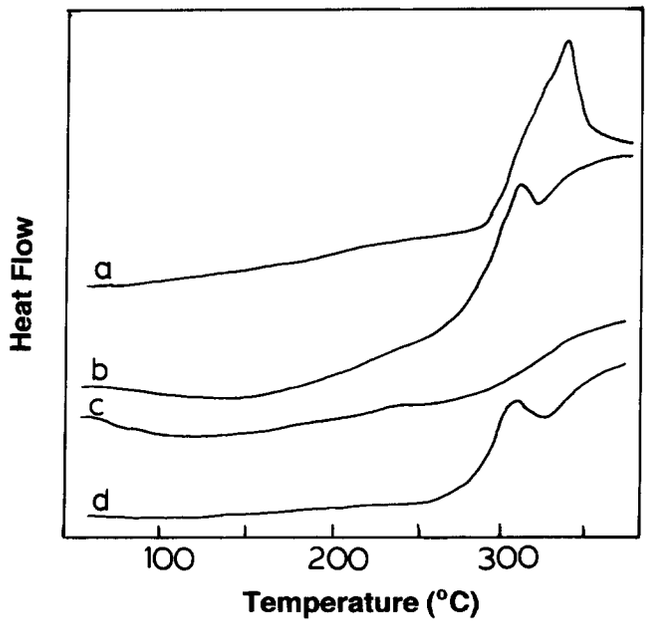

Figure 5 D.s.c. thermograms of (a) chitosan, (b) *N*-benzylidene chitosan, (c) *O*-diethylaminoethyl-*N*-benzylidene chitosan, and (d) *O*-diethylaminoethyl chitosan

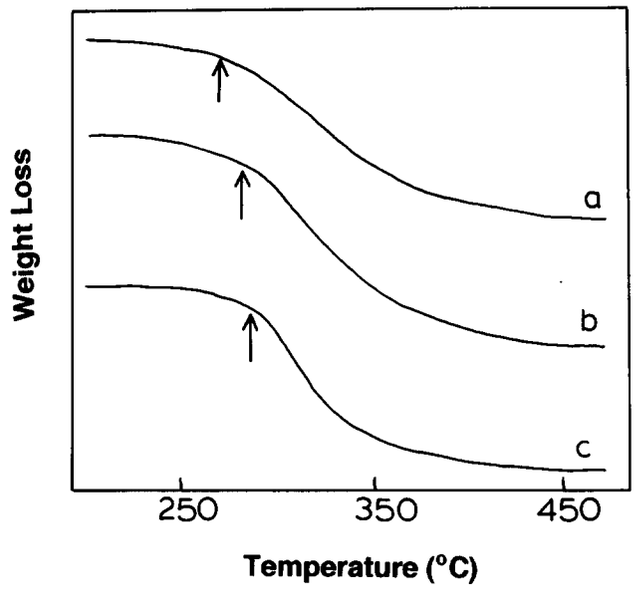

Figure 6 T.g.a. thermograms of crosslinked DEAE-chitosan membranes: (a) G-1, (b) G-2, (c) G-3

Mechanical properties

The tensile strength and the elongation at break for dry and wet samples of crosslinked DEAE-chitosan film are listed in *Table 1*. In general, it is known that the segmental mobility in the polymer chain influences the mechanical properties. Also a polymer chain having a bulky side-group experiences a restriction in rotation due to steric hindrance, resulting in increases in T_g and rigidity[12]. The DEAE-chitosan exhibited an increased tensile strength and decreased elongation at break in both dry and wet states, compared with chitosan. These results can be interpreted in terms of the above-mentioned restricted rotation of the bulky side-chain.

For crosslinked DEAE-chitosan membranes, the tensile strength increased with the amount of crosslinking agent in dry and wet states. Crosslinking apparently contributed to the formation of a three-dimensional network structure.

Swelling characteristics

Swelling kinetics of the membrane are shown in *Figure 7*. It is clear that the more acidic the solution, the faster are the swelling rates. All the samples in a different pH range reached an equilibrium state within 1 h.

The effects of crosslinking and the pH of the solution on the equilibrium degree of swelling for DEAE-chitosan membranes are shown in *Figure 8*. The equilibrium degree of swelling decreases with concentration of crosslinking agent in the membrane, mainly owing to the growing crosslink density of the polymer chains. Moreover, as the concentration of crosslinking agent increases, more amino groups in DEAE-chitosan are consumed due to the crosslinking reaction. It is well known that amino groups form a Schiff base by reacting with aldehydes. Therefore, as chitosan reacts with glutaraldehyde, crosslinked DEAE-chitosan becomes less capable of hydrogen bonding with water molecules owing to the intermolecular and intramolecular crosslinking caused by the formation of a Schiff base, resulting in a decreased degree of swelling at equilibrium. The increase in degree of swelling of DEAE-chitosan membrane with decrease of pH in solution is due mainly to the presence

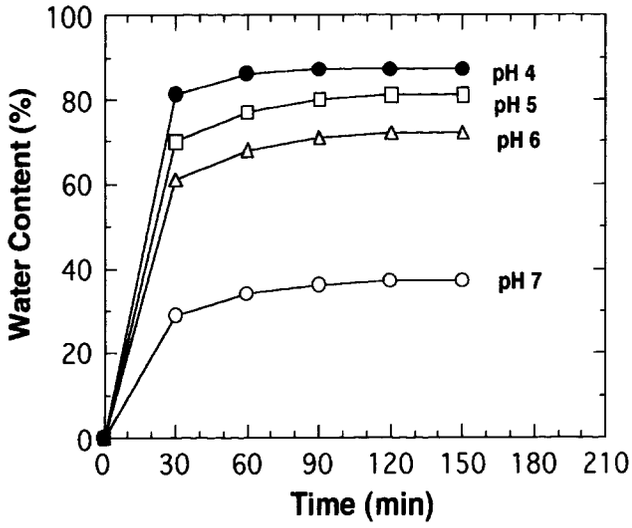

Figure 7 Swelling kinetics of G-2 membrane in the dry state at different pH values

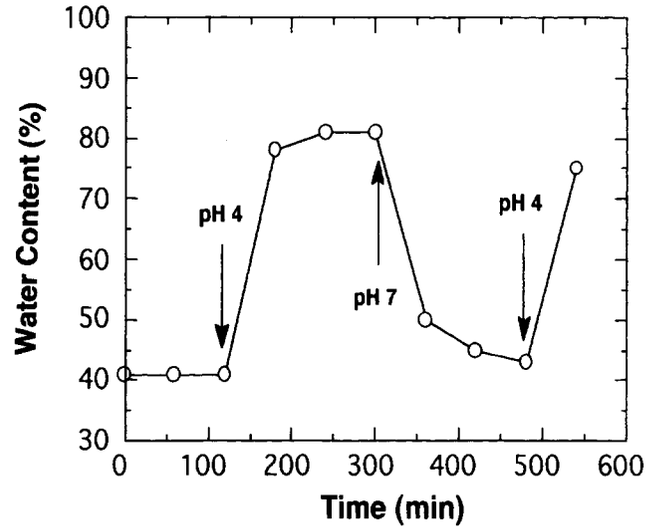

Figure 9 Swelling dynamics of G-2 membrane in the wet state

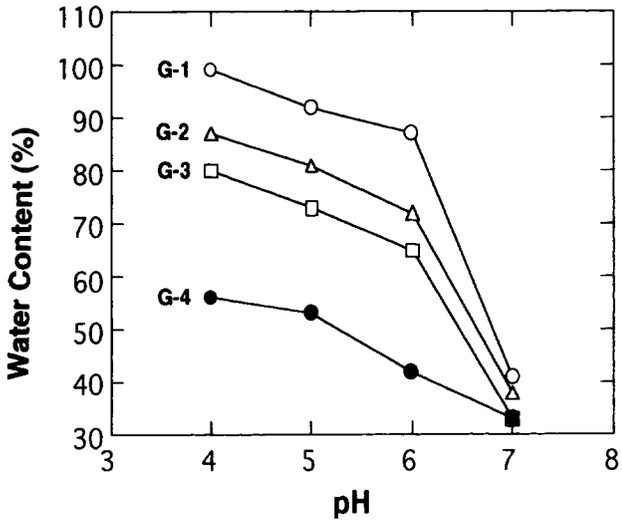

Figure 8 Effect of pH on the swelling of DEAE-chitosan membranes

of amino groups and DEAE groups in chitosan. In a low-density ionic environment and as pH is lowered, chitosan molecules become uncoiled and assume more elongation or exist in a rod-like shape. The mutual repulsion of charged groups supplies the uncoiling force. In G-1 and G-2 samples, when the pH is decreased from pH 7 to pH 6, an abrupt transition in swelling occurs with the degree of swelling rising more than about 20%. It has been reported[13] that the pK_a value of the amino groups and DEAE groups in chitosan is 6.3. A subsequent decrease in pH causes a further increase in the equilibrium degree of swelling inside the membrane.

Dynamic response of swelling to a step change in pH is illustrated in *Figure 9*. In our experiment, DEAE-chitosan membranes were brought into a swelling equilibrium at pH 4 for 1 h, and then transferred to a buffer solution at pH 7 so that an abrupt deswelling was ensured. Later the membrane was placed back into a buffer solution at pH 4, and then reswollen again for approximately 30 min. This process was repeated several times, with virtually identical kinetics being displayed in each cycle. The results demonstrated that the DEAE-chitosan membrane changes its ability to absorb water when the environmental pH is altered. The swelling transition is appealing since it implies that water permeability, which should increase with membrane hydration, can also be converted in response to a change in environmental pH. This would be a desirable characteristic for a pH-sensitive controlled-release system, because crosslinks oppose expansion of the membrane and reduce the efficiency of the blend membrane to equilibrium degree of swelling. On these grounds, pH sensitivity of the present DEAE-chitosan membrane can be changed by controlling the concentration of crosslinking.

CONCLUSIONS

Diethylaminoethyl chitosan (DEAE-chitosan) was synthesized via Schiff-base reaction between amino groups in chitosan and benzaldehyde. After reaction with diethylaminoethyl chloride hydrochloride and benzylidene chitosan (B-chitosan), the Schiff base was removed by treating the chitosan derivatives with dilute hydrochloride ethanolic solution. The tensile strength of DEAE-chitosan was improved by incorporation of the DEAE groups in the chitosan. The degree of swelling of DEAE-chitosan membrane decreased with the pH in the solution. The equilibrium degree of swelling decreases with the concentration of the crosslinking agent. The present membrane shows pH-sensitive swelling characteristics that would be applicable to a controlled-release system.

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