Dissolving States of Cellulose and Chitosan in Trifluoroacetic Acid

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SYNOPSIS

Chemical structures of cellulose and chitosan dissolved in trifluoroacetic acid (TFA) and those of cellulose and chitosan films cast from their TFA solutions were studied by ^{13}C-NMR and infrared (IR) spectroscopy. Cellulose is trifluoroacetylated selectively at the C6-hydroxyl groups in the TFA solution, and chitosan is dissolved in TFA by forming amine salts with TFA at the C2-amine groups. IR analyses of cellulose films cast from its TFA-acetic acid solutions showed that partly trifluoroacetylated cellulose in the solution state turns to partly acetylated cellulose in the solid state during evaporation of the solvents in air by the ester interchange. Chitosan films cast from its TFA-acetic acid solutions still have the amine salts with TFA. These acetyl groups in cellulose films and TFA in chitosan films are removable by soaking the films in 1*N* NaOH at room temperature for 1 day.

INTRODUCTION

Various kinds of nonaqueous solvents for cellulose have been found and investigated in terms of the dissolving states: chloral-*N*,*N*-dimethylformamide (DMF),[1] N_2O_4–DMSO,[2] paraformaldehyde–dimethylsulfoxide (DMSO),[3] *N*-methyl morphorine-*N*-oxide–DMSO,[4] SO_2–amine–DMSO,[5] and so on. In all cases, cellulose is dissolved by forming complexes or derivatives, which are unstable under aqueous conditions.

Trifluoroacetic acid (TFA) is one of the nonaqueous solvents for cellulose, and can dissolve both native and regenerated celluloses at room temperature within a few days. Furthermore, although most of nonaqueous cellulose solvent systems consist of a nonvolatile solvent such as DMSO or DMF, volatility of TFA is especially a characteristic point in the cellulose solvent systems. Patel and Gilbert[6] found that a concentrated solution of cellulose in TFA–dichloromethane showed a cholesteric-type lyotropic mesophase. During dissolution of cellulose acetate into TFA, two reactions took place on free hydroxyl and acetoxyl groups of cellulose acetate. One is trifluoroacetylation on free hydroxyl groups in cellulose acetate, and another is an ester interchange from acetyl groups to trifluoroacetyl groups.[7]

In our previous study[8,9] of preparing and characterizing cellulose–chitosan blend films, the following results were obtained: (1) TFA gave clear solutions of cellulose and chitosan mixtures at any ratios of the two polysaccharides, (2) blend films were obtained by casting the solution on a glass plate and treating the dry films with 1*N* NaOH solution, (3) the blend films were transparent, and had both sufficient strength and flexibility without adding softening agents in the films, and (4) the presence of intermolecular interactions between the two polysaccharides in the films were suggested from the results of X-ray diffraction analysis, Raman analysis, and measurements of physical properties of the blend films.

The purpose of the present study is to clarify the dissolving states of cellulose and chitosan in trifluoroacetic acid and changes of chemical structures of cellulose and chitosan during the preparation of the blend films.

EXPERIMENTAL

Materials

Microcrystalline cellulose powder (Whatman CF-11) and purified cotton were used as cellulose sam-

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ples. Two kinds of low-molecular weight celluloses ($DP_n \simeq 7$ and 15) were prepared from microcrystalline cellulose powder using 83% phosphoric acid, and were used as cellulose models.[10] Two commercial chitosans were purified by dissolution in 0.5% acetic acid, precipitation of the solution in a dilute NaOH solution, and washing the regenerated chitosan sufficiently with water. One chitosan sample (100L, Katokichi Co. Ltd., Kagawa, Japan) was used for NMR study, and another one (PSH, Yaizu Suisan Kagaku Co. Ltd., Shizuoka, Japan) for film preparation and IR studies.

Dissolution of Cellulose and Chitosan in TFA

Cellulose/TFA solutions for NMR study were obtained as follows: Cellulose (DP $\simeq$ 15, 200 mg) was completely dissolved in TFA (5 mL) at room temperature. The solution was concentrated to about 1 mL by evaporation at room temperature. Deuterated DMSO (1 mL) was then added to the solution, and it was subjected to NMR studies. Cellulose was confirmed to be stable without hydrolysis in the TFA–DMSO solution at room temperature during the ^{13}C-NMR measurement.

Chitosan solutions in TFA–DMSO-d_6 and TFA–CD_3COOD for the NMR study were prepared by a method similar to that of the cellulose solutions. Also a chitosan solution in 10% CD_3COOD–D_2O was prepared.

Cellulose and chitosan films were prepared according to the method reported in the previous paper. Namely, cellulose and chitosan were dissolved in TFA at room temperature, and acetic acid was added to the solution to control the viscosity for casting. After completely clear solutions were obtained, the solution was cast on a glass plate, and dried in a hood to remove trifluoroacetic acid and acetic acid. The dry film then was soaked in 1*N* NaOH solution for 1 day at room temperature, and washed sufficiently with water. The wet film was placed between a metal plate and filter paper sheets, and dried at 20°C and 65% relative humidity.

Analyses

^{13}C-NMR spectra were obtained with a Bruker AC-300 NMR spectrometer at 75.4 MHz. Spectra were accumulated using a 5 μs pulse width, a 18.5 kHz sweep width, and a 2 s pulse interval. All spectra were recorded at 300 K. The chemical shifts were measured on the δ scale (ppm) relative to tetramethylsilane in DMSO-d_6 and 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt in D_2O as internal standards.

Infrared (IR) spectra were recorded on a Shimadzu IR-435 spectrometer. Thin films of cellulose and chitosan were prepared according to the method of preparing the films with and without the alkali treatment, and were subjected to IR analysis after being dried under a reduced pressure.

Degrees of esterification of cellulose films were estimated by means of the semimicro method reported by Lin and Schuerch.[11]

RESULTS AND DISCUSSION

Cellulose and Chitosan in TFA

^{13}C-NMR spectra of cellulose (DP $\simeq$ 7) in DMSO-d_6 and cellulose (DP $\simeq$ 15) in TFA–DMSO-d_6 are shown in Figure 1. In the case of cellulose in DMSO-d_6, six resonances corresponding to the carbons of an anhydroglucose residue were observed with a good resolution, and were assigned on the basis of the spectra of low-molecular weight celluloses in hot DMSO-d_6[4] and in D_2O.[12] ^{13}C-NMR spectrum of cellulose in TFA–DMSO-d_6 also had six main resonances, although significant differences between the two spectra were observed at the resonances due to C5 and C6. The chemical shifts of carbons of an anhydroglucose residue of cellulose in the two spectra were summarized in Table I.

In the spectrum of cellulose in TFA–DMSO, the main signals due to C6 and C5 were located at 67.3 and 71.7 ppm, respectively, although signals due to other carbons appeared at the same chemical shifts between the two spectra. When ^{13}C-NMR spectra of ethanol and *i*-propanol in TFA–DMSO-d_6 were compared with those in DMSO-d_6, resonances due to the α-carbons having trifluoroacetoxyl groups were shifted to 7.9 and 10.7 ppm downfields for ethanol and *i*-propanol, respectively. Simultaneously, resonances due to the β-carbons were shifted to 4.8 and 4.4 ppm upper fields for ethanol and *i*-propanol, respectively. These changes of resonances due to α- and β-carbons by trifluoroacetylation of hydroxyl groups corresponded well to those due to C6 ($\Delta = -7.1$ ppm) and C5 ($\Delta = +3.0$ ppm), respectively, of cellulose in TFA–DMSO-d_6. In addition to the information about these hydroxyl carbons of ethanol and *i*-propanol, carbonyl carbons and CF_3-carbons of TFA appeared as quartets at 159.6 and 116.1 ppm, respectively, for free TFA, and as those at 157.3 and 115.3 ppm, respectively, for esterified TFA. In the case of cellulose in TFA–

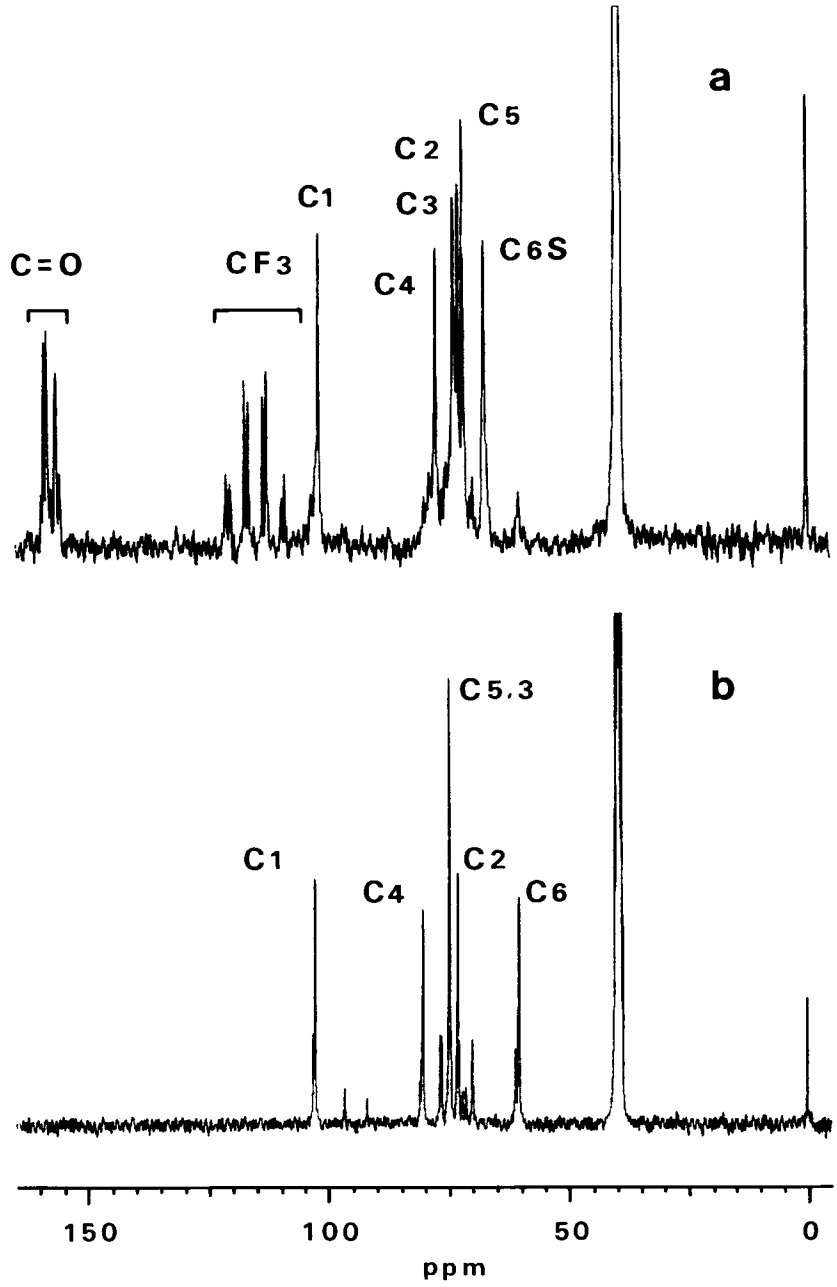

Figure 1 ^{13}C-NMR spectra of cellulose solutions in trifluoroacetic acid–DMSO-d_6 (a) and in DMSO-d_6 (b).

DMSO, two types of carbons due to TFA were observed in the spectrum, 159.6 and 115.9 ppm for free TFA and 156.6 and 114.5 ppm for esterified TFA. Even though a resonance due to C6 having free hydroxyl groups was slightly detected at 60.2 ppm in the spectrum of cellulose in TFA–DMSO, a quantitative integration of the two resonances showed that more than 85% of the hydroxyl groups at C6 of cellulose are trifluoroacetylated in TFA–DMSO under the conditions. Furthermore, since the predominant resonances due to C2 and C3 of cellulose in TFA–DMSO had almost equal chemical shifts to those in DMSO, most of secondary hydroxyl groups of C2 and C3 must be present as the hydroxyls without being trifluoroacetylated in TFA–DMSO. Therefore, these results show that cellulose is dissolved in TFA by forming trifluoroacetyl esters selectively at the C6 hydroxyl groups. This esterifi-

Table I Chemical Shifts of Carbons of Cellulose and Chitosan in Solutions

Carbons	Cellulose in TFA–DMSO	Cellulose in DMSO	Chitosan in TFA–CD_3COOD	Chitosan in CD_3COOD–D_2O
C1	102.7	101.8	98.15	100.5
C2	72.9	72.6	56.17	58.7
C3	74.7	73.7	70.70	73.0
C4	80.2	77.3	77.94	79.4
C5	74.7	71.7	75.27	77.7
C6	60.2	67.3 + (60.2)	60.79	62.9

cation of C6 hydroxyls was observed also for cellulose acetate in TFA.[7]

^{13}C-NMR spectra of chitosan in 50% TFA–CD_3COOD and 10% CD_3COOD–D_2O are shown in Figure 2. In both spectra, six resonances corresponding to the carbons of an anhydroglucosamine residue of chitosan were observed with a clear resolution. These resonances were assigned in comparison with the spectrum of 95% deacetylated chitosan in D_2O.[13] No remarkable difference of chemical shifts due to carbons was observed between the chitosan solution in TFA–CD_3COOD and that in CD_3COOD–D_2O. On ^{13}C-NMR spectrum of the chitosan solution in TFA–CD_3COOD, only one type of resonance corresponding to TFA carbons was observed as quartets at 115.7 and 158.9 ppm due to CF_3 and carbonyl carbons, respectively. As described previously, these resonances were assigned to free TFA. Therefore, the dissolving state of chitosan in TFA is similar to that of chitosan in acetic acid, and chitosan is dissolved in TFA by forming the amine salts with TFA at the amine groups of C2 in chitosan. Furthermore, most of hydroxyls at C3 and C6 of chitosan were found to be present as hydroxyls without forming trifluoroacetate in the solution state. ^{13}C-NMR spectrum of chitosan in TFA–DMSO-d_6 also was measured, but the resonances due to the carbons of chitosan were completely identical to those in TFA–CD_3COOD.

Thus, ^{13}C-NMR spectroscopic studies revealed that cellulose and chitosan are dissolved in TFA with different states; selective trifluoroacetylation at primary hydroxyl groups C6 for cellulose and the amine-salt formation with trifluoroacetic acid at C2-amino groups for chitosan. The reasons for the selective trifluoroacetylation of C6 hydroxyl groups in cellulose; in other words, the reasons for no trifluoroacetylation of C2 or C3 hydroxyl groups in cellulose, and those for no trifluoroacetylation of hydroxyl groups in chitosan have not been answered in this study.

Cellulose and Chitosan in Cast Films

In previous papers, cellulose–chitosan blend films were prepared with two steps; first the TFA–acetic acid solution of cellulose and chitosan was cast on a glass plate, and a dry film (film without alkali treatment) was obtained by evaporation of the solvents. Second the film then was soaked in 1*N* NaOH solution, and a dry film (film with alkali treatment) was obtained by washing the film sufficiently with water and by drying it in a conditioned room. In this section, the chemical structures of cellulose and chitosan in the cast film with and without the alkali treatment were studied on the basis of IR spectra of the films and the results obtained by the ^{13}C-NMR studies of cellulose and chitosan solutions in TFA. IR spectra of cellulose and chitosan films with and without the alkali treatment are shown in Figure 3.

The IR spectrum of the cellulose film with the alkali treatment (b in Fig. 3) showed a typical pattern of cellulose. In contrast, the IR spectrum of the cellulose film without the alkali-treatment (a in Fig. 3) had an absorption band in the carbonyl region around 1745 cm^{-1}. This absorption was ascribed to C=O stretching vibrations of acetyl ester groups, and no absorption bands due to carbonyls of trifluoroacetyl ester groups around 1790 cm^{-1} were detected. Furthermore, if trifluoroacetyl groups are present in the cellulose film (a), three absorption bands due to stretching vibrations of C—F should appear around 840–720 cm^{-1}, as observed in the IR spectrum (c) in Figure 3. Thus, the cellulose film without the alkali treatment contained acetyl ester groups, which originated from acetic acid added to the cellulose solution in TFA for controlling the viscosity. Degree of substitution of acetyl groups in the cellulose film (a) was calculated from the acetyl content of the film, and was 0.34. On the other hand, when a cellulose film was prepared from a cellulose/TFA solution, trifluoroacetyl ester groups were detected in the IR spectrum of the cellulose film with-

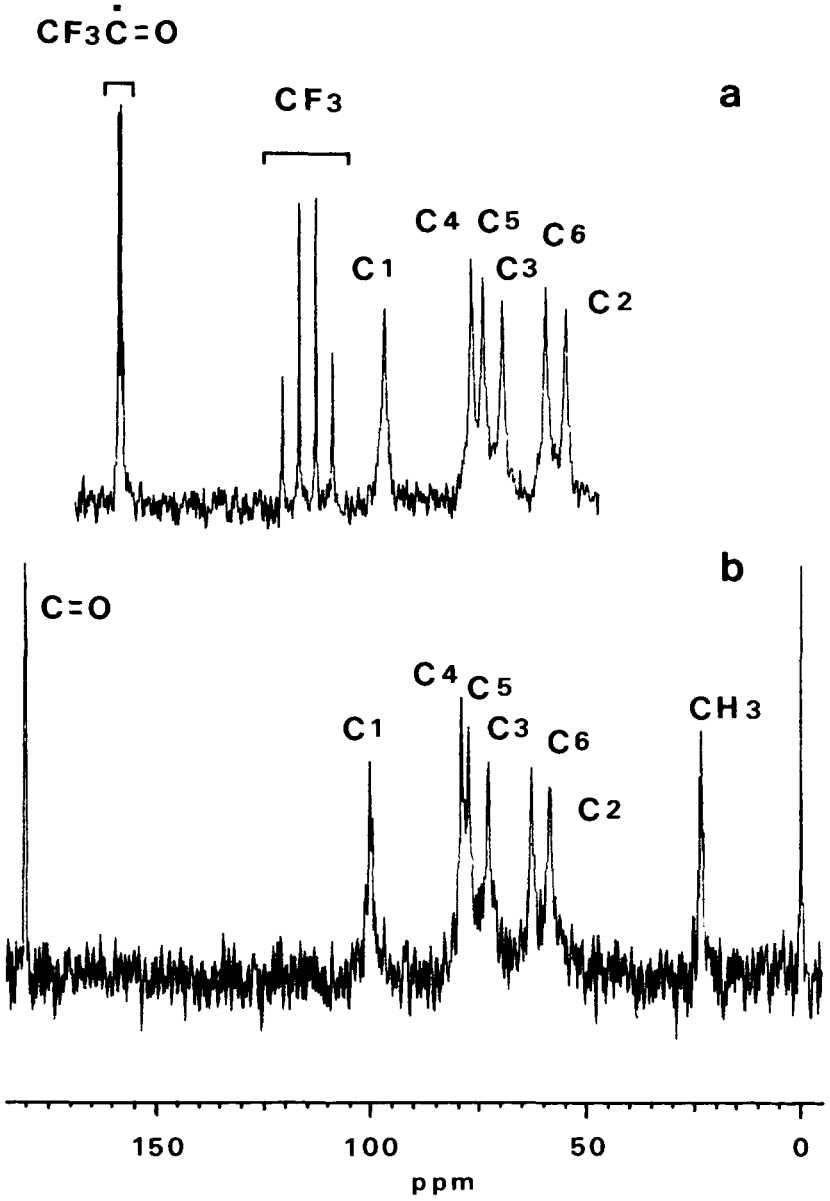

Figure 2 ^{13}C-NMR spectra of chitosan solutions in ca. 50% trifluoroacetic acid–acetic acid-d_4 (a) and in ca. 10% acetic acid-D_2O (b).

out the alkali treatment. Degree of substitution of trifluoroacetyl groups in the cellulose film was 1.1 at the initial stage, and was decreased with increasing time for exposing the film in the air. Trifluoroacetyl ester groups in the cellulose film must be gradually hydrolyzed with moisture in the air, and TFA thus formed is removed from the film by natural evaporation.

These observations together with those by ^{13}C-NMR analyses support the idea that the hydroxyl groups of C6 in cellulose dissolved in TFA are selectively esterified by TFA, and then the trifluoroacetyl ester groups are partly converted to acetyl ester groups by the ester interchange in the TFA–acetic acid mixture during evaporation in air. Trifluoroacetyl ester groups in cellulose are unstable in air and are hydrolyzed with moisture, whereas acetyl ester groups once formed in cellulose are stable to moisture in air.

On the other hand, IR spectra of the chitosan film without the alkali treatment had no absorption bands due to acetyl ester or trifluoroacetyl ester groups. The large absorption band around 1670 cm^{-1} and three absorption bands around 840–720 cm^{-1}

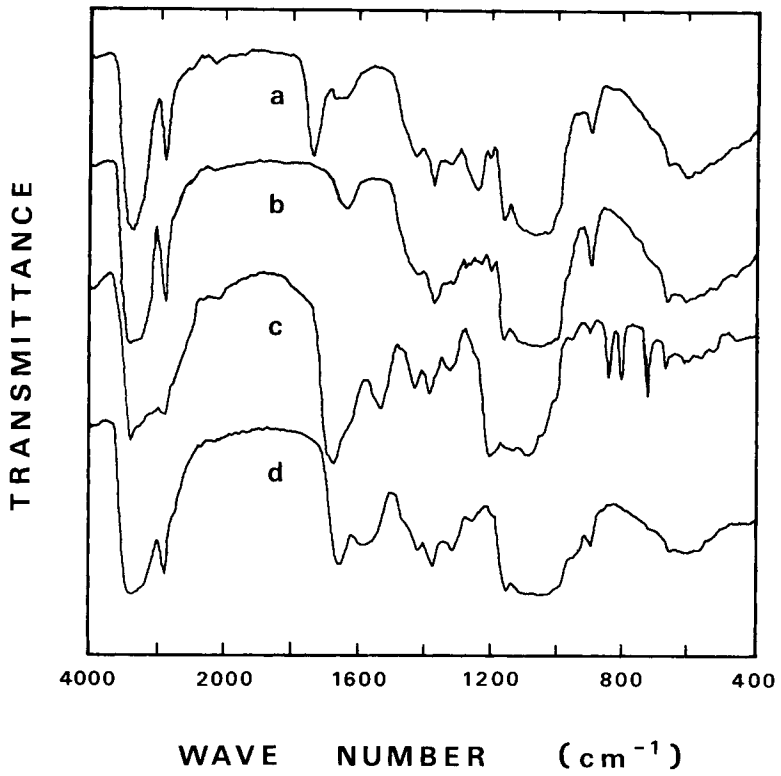

Figure 3 IR spectra of cellulose and chitosan films prepared from their trifluoroacetic acid–acetic acid solutions, with and without 1*N* NaOH treatment: (a) cellulose film without alkali treatment; (b) cellulose films with alkali treatment; (c) chitosan film without alkali treatment; (d) chitosan film with alkali treatment.

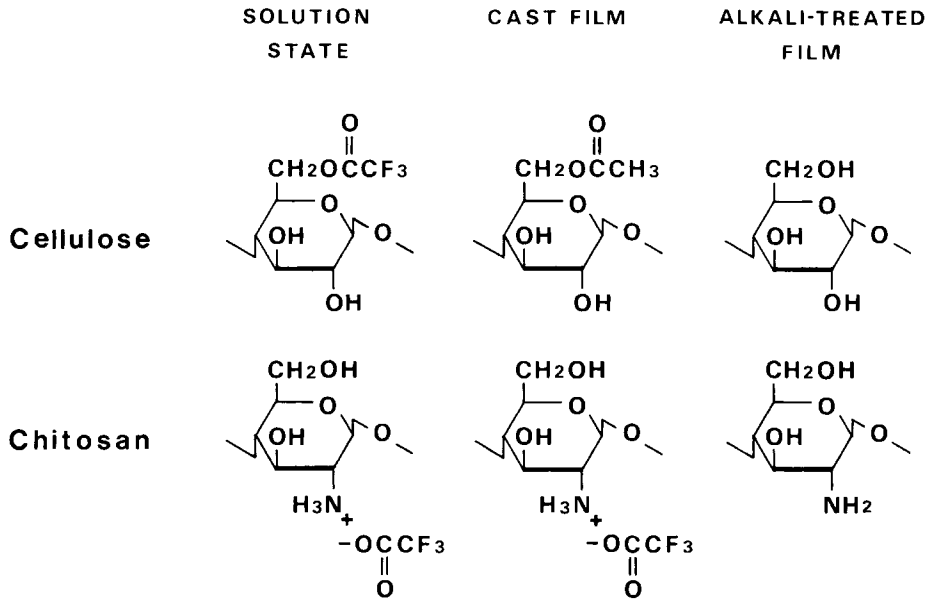

Figure 4 Chemical structures of cellulose and chitosan in trifluoroacetic acid, in cast films without alkali treatment and in cast films with alkali treatment.

showed the presence of trifluoroacetic acid in the chitosan film as amine salts. These absorption bands due to TFA in the chitosan film disappeared by the alkali treatment of the film, as shown in the IR spectrum d in Figure 3. The absorption band around 1640 cm^{-1} is ascribed to carbonyls of amide groups originally present in chitosan, whose degree of deacetylation was about 80%.

Therefore, cellulose and chitosan are dissolved in TFA by forming trifluoroacetyl ester groups at the hydroxyls of C6 and the amine salts with TFA at C2, respectively. When cast films are prepared from cellulose–chitosan solutions in TFA–acetic acid, cellulose and chitosan have acetyl ester groups and amine salts with TFA, respectively, in the blend films without the alkali treatment. These acetyl ester groups of cellulose and the amine salts of chitosan were removed by the alkali treatment, and cellulose with free hydroxyls and chitosan with free amine groups are formed in the blend films (Figure 4).

CONCLUSIONS

Cellulose molecules are dissolved in TFA by forming trifluoroacetyl ester groups almost selectively at the primary hydroxyl groups C6 in an anhydroglucose residue. Chitosan molecules form amine salts at the amino groups of C2 with TFA in an anhydroglucosamine residue with in the TFA solution, and no trifluoroacetylation occurs on hydroxyl groups of chitosan.

Cellulose molecules in films cast from cellulose/TFA–acetic acid mixture are partly acetylated at probably C6 hydroxyls by the ester interchange from trifluoroacetyl groups to acetyl groups in the presence of acetic acid during the drying process. The amine salts of chitosan in TFA is still present in the film without the alkali treatment. The acetyl groups in cellulose and the trifluoroacetic acid in chitosan are completely removed by soaking the films in 1*N* NaOH solution.

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