Simple Determination Method of Degree of Substitution for Starch Acetate

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To develop a simple method to determine the degree of substitution (dS) for starch ester, we prepared starch acetate with various dS values (0—3) and examined the mode of starch-iodine complex formation for the starch acetate by monitoring the ultraviolet-visible (UV-vis) spectra and their Fourier-transform infrared (FT-IR) spectral changes. The effect of acetylation on the formation of the starch-iodine complex was investigated by monitoring the decrease in absorbance at 680 nm (blue value). The blue value decreased by increasing the standard dS value determined by NMR for starch acetate, but two different correlations between the blue values and the dS values were observed for the low- and highly-substituted starch acetate. The FT-IR spectra for starch acetate particles were measured using a diffuse reflection method. The peak areas and absorbances of the bands assigned to the acetyl group showed a poor correlation with standard dS values from the NMR. On the other hand, the FT-IR peaks assigned to sodium acetate produced by saponification of the starch acetate could be detected using the ATR (attenuated total reflection) method; they showed a good correlation with the standard dS values from the NMR. From these results, it was concluded that the ATR method should be available for the dS determination of starch acetate.

Starch has been widely used in the paper manufacture and cotton spinning industries, but retrogradation of the starch gel occurs during storage at low temperature. We previously reported that the retrogradation of starch could be protected by the addition of hydrogen bond-breaking materials, such as guanidine hydrochloride.[1,2] Also, the degree of retrogradation can be restrained by some modifications of the starch. For example, starch acetate has been used as a food additive in the food industry because of its retrogradation resistance, food safety, and economic stabilization. Starch acetate can be prepared by the reaction of starch with acetic acid,[3] acetic anhydride,[4,5] vinyl acetate,[6] acetyl chloride, and diketene, and can be triesterified. The availability of starch acetate depends on the degree of substitution (dS).

The percentage and degree of substitution express the degree of esterification. The percentage of substitution is the weight percentage of the substituent, and the dS value gives the average number of substituted hydroxy groups per anhydroglucose unit. The quantitative analysis of the dS for starch acetate has been performed by the following methods. Methyl acetate formed from the treatment of the starch acetate with sodium methoxide in absolute methanol can be analyzed using a gas chromatograph.[7] In a titration method, the amount of acetate produced by saponification of the starch acetate is also determined.[8,9] These two methods are indirect dS determination techniques that require some treatments. On the other hand, it is possible to quantitatively analyze the absorption band (1724 cm^{-1}) assigned to the C=O stretching vibration of the ester group using infrared (IR) spectroscopy.[10] Recently, another direct method to determine the dS using NMR has been developed.[11] However, the titration method with the saponification has been generally used for the dS determination due to the easy handling without specific instrumentation such as IR and NMR spectrometers.

For other modified starch besides starch acetate, various quantitative analytical methods of the substituents have also been reported.[12—15] However, each method was specific to its substituent and was individual for all the modified starch. In the present study, to develop a simple evaluation method of the dS value for the starch ester, we examined the various spectral changes of starch acetate with the increasing dS value. FT-IR and UV-vis spectroscopies have been examined as a dS determination method for starch acetate. IR peaks assigned to sodium acetate produced by the saponification of starch acetate using attenuated total reflection (ATR) were linearly correlated with the standard dS values obtained from the NMR method. The FT-IR measurement using the ATR technique is available to the dS determination. In addition, the effect of esterification on the starch-iodine complex formation was estimated by monitoring the absorbance change

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at 680 nm (blue value). In the low dS range (dS < 0.2), a good correlation between the blue values and the standard dS values from the NMR method was observed, suggesting limited use of this UV-vis method for the dS determination.

Experimental

Reagents. Corn starch and acetic anhydride (analytical grade) were purchased from Honen Co. and Kanto Chemical Co., respectively.

Syntheses of Starch Acetate. Starch acetate was synthesized by two methods using water and pyridine as the reaction solvents. In the first method using water, 50 g of corn starch was dispersed into 150 mL of distilled water at 37 °C in a 300-mL beaker. The appropriate amount of acetic anhydride (5—12 g) was added dropwise into the starch suspension while the pH was kept between 10—11.5 by the addition of 4% NaOH. The mixture was stirred at 37 °C for 1 h, and then neutralized with dilute sulfuric acid. After filtration, the obtained filter cake was dispersed in 500 mL of water. To thoroughly wash the product, this washing was repeated twice. Finally, one more filtration was carried out with 250 mL of methanol; then the filter cake was dried under reduced pressure. This method gave only low-substituted starch acetate (dS < 0.2).

To prepare highly-substituted starch acetate (dS > 0.2), the corn starch was gelatinized before acetylation by another method.[5] Fifty grams of corn starch was dispersed into 550 mL of distilled water in a 1000-mL round-bottom flask. The suspension was gelatinized by stirring at 100 °C for 20 min in a water bath. The gelatinized starch was precipitated from ethanol, filtered, and dried under reduced pressure after washing with acetone. The obtained gelatinized-starch (25 g) was dispersed in 200 g of pyridine in a 500-mL round-bottom flask. To prepare starch acetate with the desired dS, appropriate amounts of acetic anhydride (10—80 g) were added to the suspension. The reaction was carried out by stirring at 100 °C for 4 h in a water bath. After cooling to room temperature, the product was precipitated from 1000 mL of ethanol. The obtained precipitate was filtered, washed well with ethanol, and dried under reduced pressure. This method gave not only highly-substituted starch acetate but also a low-substituted one (dS < 0.2) by changing the amount of acetic anhydride. However, all the results were independent of the difference between the two methods for the synthesis of starch acetate.

Measurement Methods for Degree of Substitution. The standard dS values for starch acetate were determined by titration combined with saponification and direct NMR methods.

Titration Method.[9] Five grams of starch acetate and 50 mL of distilled water were dispersed in an Erlenmeyer flask with a stopper. Phenolphthalein was added to the suspension as an indicator, and then a 0.1-mol dm^{-3} NaOH solution was added to retain the red color. After the addition of 25 mL of 0.45 mol dm^{-3} NaOH solution, the mixture was stirred at room temperature for 30 min. The flask was corked to prevent evaporation of the produced acetate during the saponification reaction. Finally, the excess alkali in the sample mixture was titrated with a 0.2-mol dm^{-3} HCl solution. A blank test was also carried out with corn starch using the same procedure.

Another titration procedure was required for complete saponification of the highly-substituted starch acetate (dS > 0.2). One gram of starch acetate and 50 mL of 75% ethanol were mixed in an Erlenmeyer flask with a stopper; then the mixture was stirred in a water bath at 50 °C for 30 min. After cooling to room temperature, this mixture was dispersed in 40 mL of 0.5 mol dm^{-3} KOH solution, and then stirred at room temperature for 48 h with a stopper for the complete saponification. An excess of alkali in the solution was then titrated with 0.5-mol dm^{-3} HCl solution. A blank test for corn starch was also carried out according to the same procedure.

The percents of the acetyl group (db) and dS were calculated using Eqs. 1 and 2, respectively. Values for the blank and sample are the volumes used in titration.

$$\mathrm{db} = \frac{(\text{value for blank} - \text{value for sample})\ (\mathrm{mL})}{(\text{sampling weight})\ (\mathrm{g})} \times \text{normality of HCl} \times 0.043 \times 100, \quad (1)$$

$$\mathrm{dS} = \frac{162 \times (\mathrm{db})}{4300 - 42 \times (\mathrm{db})}. \quad (2)$$

^{1}H NMR Spectrometry.[16] ^{1}H NMR spectra were measured with a JEOL-JMN-A400 spectrometer operating at a ^{1}H frequency of 399.65 MHz. All of the chemical shifts were reported in parts per million (ppm) using tetramethylsilane as the internal standard. The sample concentrations were 6 mg ml^{-1} in DMSO-d_6 and the samples were gelatinized by stirring at room temperature for 40 h. The spectra were acquired at 80 °C with 64 accumulations to avoid an overlap of the signals for water and the hydroxy groups of the starch acetate.

X-Ray Diffraction. X-Ray diffraction analyses were performed with a Rigakudenki AC-1C powder X-ray diffractometer operating at 40 kV and 20 mA with CuK radiation. Diffractograms were recorded from 2° to 30° (diffraction angle, 2θ) at a scanning rate of 2° min^{-1}.

UV-vis Measurements. The blue values (absorbance at 680 nm) for the starch-iodine complex were measured using a Shimadzu UV-2100S recording spectrophotometer with a 10-mm cell.[17] All samples used for these UV-vis measurements were prepared as follows: Starch acetate (2 mg) and 10 mL of distilled water were mixed in a 100-mL round bottom flask equipped with a condenser and a drying tube. After the mixture was stirred at 100 °C for 30 min, an iodine solution composed of 40 mL of distilled water, 4 mg of iodine, and 40 mg of potassium iodine was added to the mixture.[17] The solution was then stirred for 30 min until the solution temperature decreased to room temp.

FT-IR Spectrometry. FT-IR spectra were measured using the diffuse reflection and ATR methods. In the diffuse reflection method, the FT-IR spectra for starch acetate particles were directly analyzed. On the other hand, the amounts of sodium acetate produced by saponification of the starch acetate were analyzed by the ATR method. Shimadzu FT-IR-8200PC and Horiba FT-710 recording spectrophotometers were used for the diffuse reflection and ATR methods, respectively.

For the diffuse reflection method, 1 mg each of starch acetate with various dS values was diluted with 7 mg of KBr and the mixture was directly applied. The number of scanning performed was 10.

Analytical samples used for the ATR method were prepared as follows: A starch acetate suspension (10%) in 2 mol dm^{-3} NaOH was gelatinized by stirring at room temperature for 24 h in a screw cap bottle (1.6 mL) to give the deacetylated starch. The obtained starch gels were applied onto the ZnSe ATR cell and the FT-IR spectra were recorded. The data acquisition was repeated 100 times. The FT-IR spectrum for native corn starch as a reference was measured using the same procedure. The difference spectrum was obtained by subtracting the absorption spectrum of the reference from that of the sample.

Results and Discussion

Determination of Standard dS for Starch Acetate.

The dS values of the prepared starch acetate were determined by titration combined with the saponification and NMR methods, as shown in Table 1. Figure 1 indicates a good correlation between the dS values from both methods. The reliability of the dS values from the NMR is higher compared to that of those from the titration method because partially ineffective deacetylation for highly-substituted starch acetate often occurs under the saponification conditions.[16] However, there is no obvious difference between the two dS values, as seen in Table 1 and Fig. 1. Therefore, the dS values from the NMR method were used as the standard dS value in this study.

Quantitative Analyses with UV-vis Measurements. Amylose forms a starch-iodine complex by adding iodine solution; consequently, its color turns blue-purple. We expected that the acetylation of starch should influence the starch-iodine complex formation and that the color change might show a correlation with the dS values for starch acetate. Thus, the blue values (absorbance at 680 nm) for the starch acetate with various dS values were compared as a function of the standard dS values from the NMR. Figure 2 shows the change in the blue value with increasing dS values. There was no simple linear correlation between the blue value and the standard dS value. The obtained plots can be approximated by two straight lines for the low- and highly-substituted dS ranges. A rapid decrease in the blue value was observed in the low dS range (0—0.2); the slope was -1.330. The decreasing rate of the blue value versus the standard dS value seems almost linear in this low dS range, i.e., the correlation coefficient was 0.999. The observed decrease should mainly result from a decay the amylose-iodine complex. These findings indicate that the formation of the amylose-iodine complex is sensitive to acetylation, which might cause the rapid decrease in the blue value. On the other hand, the decrease in blue value above dS 0.2 becomes relatively slow; the slope was -4.203×10^{-2}. The correlation coefficient (0.837) between the blue values and the standard dS values was poorer than that in the low dS range (see above). The two approximate lines intersect at dS 0.212, as shown in Fig. 2, indicating the mode of the starch-iodine complex formation change in this dS range.

There is a possibility that the two different observed correlations may be attributed to the different structural properties between the low- and highly-substituted starch acetate. This assumption comes from the fact that the low- and highly-substituted starch acetate were prepared by two distinct procedures in which the acetylation was done without and with the gelatinization of starch, respectively.

To examine the effect of the preparation procedures on the blue value changes, a low substituted starch acetate was prepared by the same procedure with the gelatinization as the high one and its blue value was compared. On the basis of the standard dS value from the NMR, the obtained blue value for the low-substituted starch acetate prepared with gelatinization was in good agreement with that prepared without gelatinization (see Fig. 2). This result indicated that the acetylation of starch should take place in a similar manner regardless of the preparation procedures and, consequently, affords no significant change in the correlation between the blue value and the standard dS. As a result, it is suggested that the UV-vis method monitoring the blue value change

Table 1. Degree of Substitution (dS) on Starch Acetate Determined by ^{1}H NMR and Titration Methods

Sample No.	dS	
	Saponification	^{1}H NMR
1	0.083	0.094
2	0.139	0.160
3	0.482	0.517
4	0.746	0.819
5	1.238	1.440
6	1.273	1.436
7	1.325	1.372
8	1.544	1.722
9	1.627	1.782
10	2.547	2.498
11	2.619	2.593

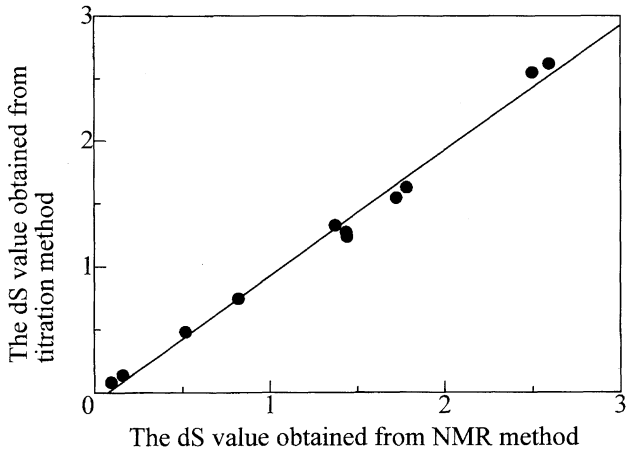

Fig. 1. Correlation between the dS (degree of substitution) values obtained from titration and NMR methods.

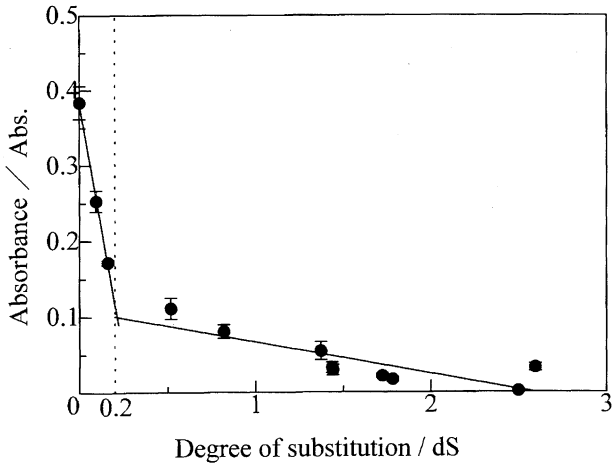

Fig. 2. Calibration curve obtained from absorbance at 680 nm in UV-vis spectra. The dS was calculated from results of ^{1}H NMR measurement.

may be available for the dS determination of starch acetate, but should be limited to the low-substituted one (below dS 0.2).

Quantitative Analyses with FT-IR Spectrometry (Diffuse Reflection Method). In the FT-IR spectra for starch acetate, two absorption bands assigned to the C=O and C–O stretching vibrations were observed in the range of 1715—1750 and 1250—1300 cm^{-1}, respectively (data not shown). Changes in the area and absorbance for these two bands with increasing dS value for the starch acetate were measured using FT-IR spectrometry (diffuse reflection method). The observed area and peak absorbance for the acetyl group as a function of the standard dS values were plotted as shown in Figs. 3 and 4, respectively, and a simple linear correlation was not observed. Two phases seem to exist in the relation between the low- and high-substitution ranges (below and above around dS 0.2, respectively). This biphasic pattern is similar to that which appeared in the UV-vis method and it is interesting that the slope changed at around dS 0.2. The correlation coefficients for the low- and high-substitution ranges are shown in Table 2. This result indicates that the peak area change shows a good correlation with the standard dS values in the both low- and highly-substituted ranges if the two approximate lines can be applied.

On the other hand, in the diffuse reflection method, a homogeneous starch acetate particle is required for the quantitative analysis because only acetyl groups on the surface of the starch particle are evaluated. However, it is difficult to prepare starch acetate particles with a homogeneous size by precipitation. This fact leads to the conclusion that this diffuse reflection method should not be used for the determination of the dS for starch acetate.

Quantitative Analyses with FT-IR Spectrometry (ATR Method). Starch ester is hydrolyzed in alkaline solution and the saponified starch ester is gelatinized at room temperature.[18] In the case of starch acetate, sodium acetate is produced in the gelatinized starch by saponification. In our preliminary experiment, the FT-IR spectrum for sodium acetate aqueous solution was measured using the ATR method and the difference spectrum was obtained by subtracting the spectrum for water. Two absorption bands assigned to the CO_2^- symmetric and CO_2^- asymmetric stretching vibrations clearly appeared in the range of 1550—1610 cm^{-1} and 1300—1420 cm^{-1}, respectively (Fig. 5a). This difference spectrum was similar to that for the saponified starch acetate gel, as shown in Fig. 5b. This result implied that the

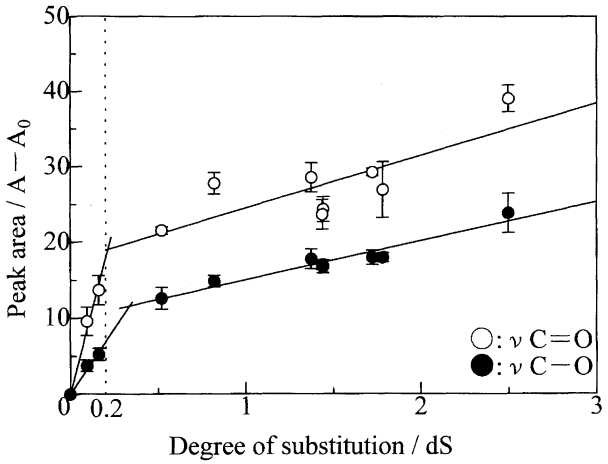

Fig. 3. Calibration curves obtained from peak areas assigned to ester in FT-IR spectra (diffuse reflection method) for starch acetate.

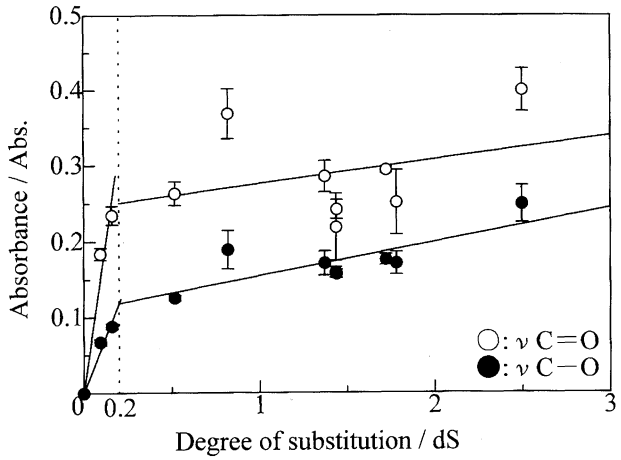

Fig. 4. Calibration curves obtained from absorbance of peaks assigned to ester in FT-IR spectra (diffuse reflection method) for starch acetate.

Table 2. Points of Intersection and Correlation Coefficients for Calibration Curves from UV-vis and FT-IR Spectroscopic Data

Method			Intersection	Correlation coefficient	
				dS < 0.2	dS > 0.2
UV-vis	Absorbance		0.212	0.999	0.837
FT-IR (Diffuse reflection)	Absorbance	νC=O	0.157	0.977	0.301
		νC–O	0.201	0.981	0.765
	Peak area	νC=O	0.212	0.992	0.790
		νC–O	0.335	0.990	0.968
FT-IR (ATR)	Absorbance	$\nu_s CO_2^-$		0.987	
		$\nu_{as} CO_2^-$		0.984	
	Peak area	$\nu_s CO_2^-$		0.991	
		$\nu_{as} CO_2^-$		0.985	

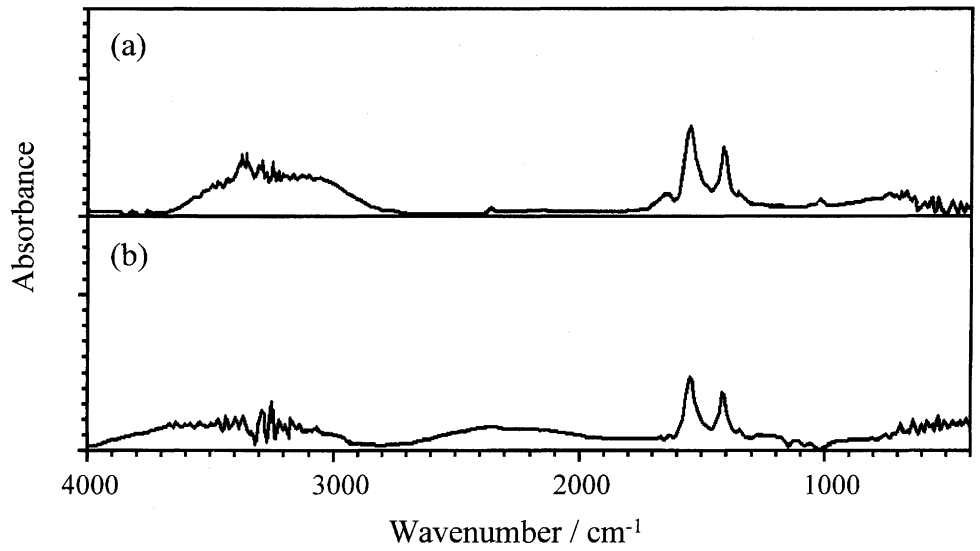

Fig. 5. Difference spectra (FT-IR) obtained by subtracting (a) H_2O from CH_3COONa solution and (b) starch gel from starch acetate gel.

ATR method was suitable for the quantitative analysis of the sodium acetate concentration.

Figure 6 shows an almost linear correlation (0.985—0.991) between the peak areas and the standard dS values in all the dS ranges. This finding suggests that there is no influence of the starch acetate structural properties on the determination of sodium acetate after the saponification by NaOH. A similar good correlation (0.984—0.987) was observed between the absorbance and the dS, as shown in Fig. 7. These results demonstrate that the ATR method is available for the determination of the dS for starch acetate. Actually, a calibration curve is required for the application of the ATR method to the dS determination for starch acetate, and moreover, the ATR method is an indirect method requiring saponification. However, there is an advantage that saponified starch acetate gels can be used for the FT-IR (ATR method) measurement without further treatment. Therefore, the ATR method should be more suitable for the dS determination for starch acetate than the other tested methods (UV-vis and diffuse reflection methods).

Crystal Structure. To investigate the effect of the acetylation of starch on the structure, crystal structures for the starch acetate with various dS values were studied using powder X-ray diffraction analysis. X-Ray diffractograms for the starch acetate were compared with that for the native corn starch as shown in Fig. 8. The starch acetate (dS 0.16), which was prepared without gelatinization process, showed a typical pattern corresponding to an A-form crystal structure, as was the case for the native corn starch. The acetylation must take place in amorphous regions of the corn starch that are mainly composed of amylose. This limited acetylation led to the maintenance of the A-form crystal structure in the starch acetate (dS 0.16). On the other hand, the A-form crystal structure disappeared in the case of starch acetate prepared via the gelatinization process (dS > 0.2). These diffraction patterns are different from A- and B-, and from their mixed forms, and should show a unique structural property of the starch acetate. During the acetylation of gelatinized starch,

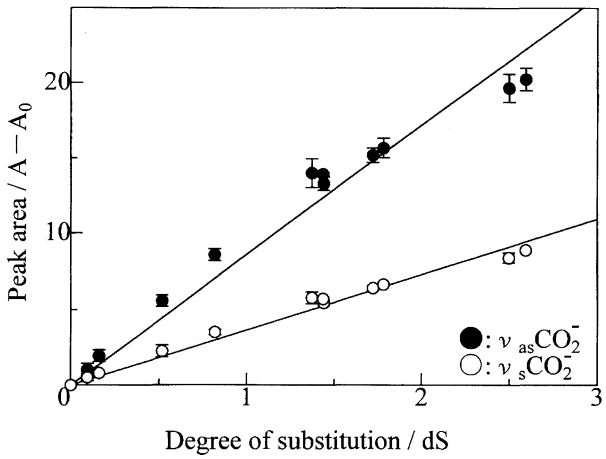

Fig. 6. Calibration curves obtained from peak areas assigned to sodium acetate in FT-IR spectra (ATR method) for saponified starch acetate.

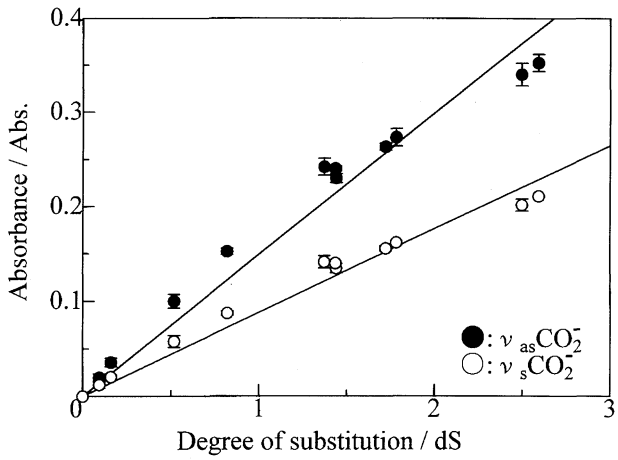

Fig. 7. Calibration curves obtained from absorbance of peaks assigned to sodium acetate in FT-IR spectra (ATR method) for saponified starch acetate.

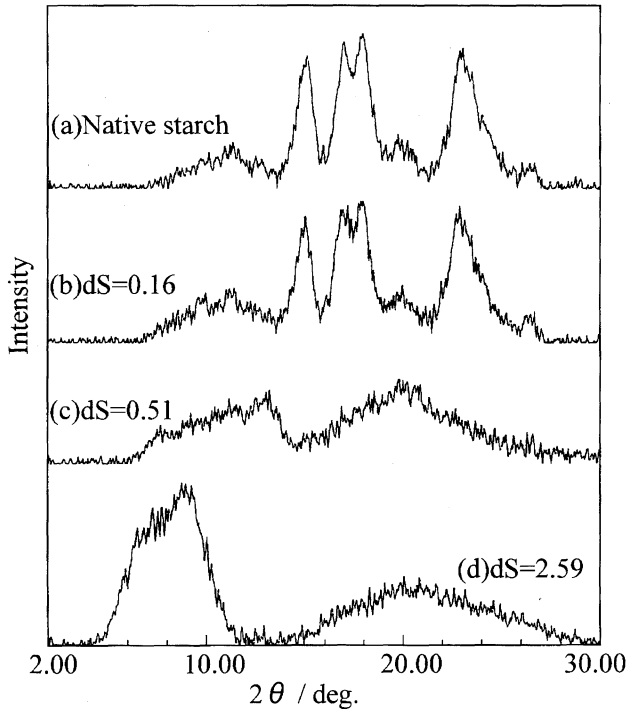

Fig. 8. X-Ray diffraction patterns for native starch and starch acetate.

most of the hydroxy groups of gelatinized corn starch are exposed to acetic anhydride because the crystal structure of starch becomes amorphous during the gelatinization process. This impartial acetylation results in the high substitution with the unique diffraction pattern.

Conclusion.

To develop a simple determination method of dS for starch acetate, the blue value change and IR spectral change (diffuse reflection and ATR methods) with increasing dS values were examined. Among the methods tested in this study, the best correlation was observed between the IR spectral change monitored using the ATR method and the standard dS value. The starch acetate gels obtained by saponification can be directly applied to the FT-IR measurement in this ATR method without further treatment. Moreover, the correlation of the IR spectral change with the standard dS value is independent of the structural change of the starch acetate. Therefore, it is concluded that the ATR method is recommended as a simple determination method of dS not only for starch acetate but also for starch ester.

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