Development of NMR and Raman Spectroscopic Methods for the Determination of the Degree of Substitution of Maleate in Modified Starches

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In this paper we report the application of NMR spectroscopy and Raman spectroscopy to determine the degree of maleate substitution in maleinated starches. Five kinds of maleinated starches were investigated and calibration sets were constructed to derive linear regression equations that may be used to predict the degree of maleate substitution for starch samples with unknown amounts of chemical modification. The calibration sets reported have very high linearity ($r > 0.99$) for both the NMR and Raman methods. The NMR and Raman calibration sets allow fast and nondestructive measurement of the degree of maleate substitution for different starches with little need of sample preparation.

Keywords: *Raman spectroscopy; maleinated starch; NMR spectroscopy; substitution degree*

INTRODUCTION

Maleinated starch is an anionic starch that may be prepared by acylation of starch with anhydrides of dicarboxylic acids to form starch esters of organic polycarboxylic acids. The level of maleination affects the physicochemical properties of the starch and hence its usefulness for different manufacturing applications (*1*). Therefore, it is important to determine and control the amount of maleate substitution in a variety of starches. Typically, wet chemistry titrimetric methods are used to find the amount of maleate substitution in maleinated starches (*2*). However, these wet chemistry methods are destructive of the starch sample, usually need time-consuming sample preparation, involve the use of hazardous chemicals that add disposal problems and costs to routine testing, and are not practical to the development of a quality control method to allow process control for manufacturing.

Because different substances have different NMR spectra (*3* or Raman spectra (*4*, *5*), their individual contributions to either a NMR or Raman spectrum can typically be discerned. Both NMR and Raman spectroscopies have been used as quantitative analytical methods in the pharmaceutical, polymer, and food industries (*3*, *5*). Recent improvements in laser sources and notch filters for stray light rejection have led to a substantial increase in the development of Raman spectroscopy for analytical applications in those industries (*6*−*20*).

In this paper, we report the development of NMR and Raman spectroscopies for the routine determination of the degree of maleate substitution in maleinated starches. We have developed calibration curves for both NMR and Raman spectroscopy that can be used to find the degree of substitution of maleate in chemically modified starches (waxy maize, regular maize, two types of high amylose maize, and wheat) with unknown degree of maleination. The NMR and Raman spectroscopy methods display very high linearity of the marker band intensities with the level of maleination of the starches, have similar limits of detection (LOD), and can obtain nondestructive measurements of the degree of substitution of maleate much faster than the currently used destructive wet chemistry titrimetric method.

MATERIALS AND METHODS

Materials. Four types of maize starches (waxy, regular, Gelose 50, and Hi-Maize) were supplied by Starch Australasia Limited (Lane Cove, Australia). These starches had the following amylose contents: 3.3% for waxy, 22.4% for regular, 47% for Gelose 50, and 66% for Hi-Maize. Wheat starch was obtained from Sigma Chemical Co. (St. Louis, MO). Maleic anhydride was purchased from Aldrich Chemical Co. (Milwaukee, WI). Maleinated starch samples were prepared by dissolving 2.5 g of sodium carbonate in 75 g of water and then suspending 50 g of starch in the alkaline solution with agitation. Maleic anhydride (varying amounts were used to achieve different degrees of substitution) was added to the stirred slurry solution. Agitation of this solution was continued for 20 h at room temperature with the pH kept at 8 using 1 N NaOH. The sample slurry was then adjusted to a pH of 7 with 0.5 N HCl, and then it was centrifuged for 5 min at 2500 rpm. The sample was then washed three times with distilled water, washed once with 95% ethanol, and finally oven-dried at 40 °C.

Determination of the Degree of Maleate Substitution by Titrimetric Analysis. The degree of substitution (DS) of maleinated starch samples was determined using the method of Wurzburg (*2*) with minor modifications. Approximately 1.0 g of dry maleinated starch sample was weighed accurately and placed in a 250-mL flask. A 75% ethanol solution in distilled water was then added, and the loosely stoppered flask was agitated, warmed to 50 °C, held at that temperature for 30 min, and then cooled to room temperature. Standard 0.200 N potassium hydroxide (20.00 mL) was added while swirling the solution; the flask was then stoppered and agitated in a shaker

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Table 1. Weight Percent and Degree of Substitution of Maleate in Modified Starches. Ratio of the Raman Intensity of the ~1641 cm^{-1} C=O Stretch and ~941 cm^{-1} C−C Stretch Bands (I_{1641}/I_{941}).

sample	weight percent maleate[a]	D. S. of I_{1641}/I_{941}	area[b] ratio I_{1641}/I_{941}	CLS[c] ratio $I_{maleate}/I_{starch}$	NMR[d] ratio
			Waxy Maize		
pure (A)	0.000 ± 0.064	0.0000 ± 0.0008	−0.0091 ± 0.0187	0.0000 ± 0.0052	0.0000
sample 1 (B)	1.273 ± 0.336	0.0171 ± 0.0045	0.1326 ± 0.0098	0.3780 ± 0.0005	0.03629
sample 2 (C)	2.241 ± 0.095	0.0303 ± 0.0013	0.2043 ± 0.0183	0.5919 ± 0.0078	0.05067
sample 3 (D)	3.083 ± 0.199	0.0421 ± 0.0028	0.2845 ± 0.0167	0.7813 ± 0.0158	0.06845
sample 4 (E)	3.848 ± 0.115	0.0529 ± 0.0016	0.3631 ± 0.0094	0.9991 ± 0.0066	0.08675
			Regular Maize		
pure (A)	−0.046 ± 0.086	−0.0006 ± 0.0011	0.0054 ± 0.0048	0.0000 ± 0.0085	0.0000
sample 1 (B)	0.818 ± 0.231	0.0109 ± 0.0031	0.0855 ± 0.0086	0.3655 ± 0.0075	0.02034
sample 2 (C)	1.408 ± 0.075	0.0189 ± 0.0010	0.1354 ± 0.0279	0.6097 ± 0.0173	0.03355
sample 3 (D)	1.965 ± 0.217	0.0265 ± 0.0030	0.1802 ± 0.0107	0.7829 ± 0.0064	0.04537
sample 4 (E)	2.680 ± 0.316	0.0364 ± 0.0044	0.2437 ± 0.0163	1.0000 ± 0.0063	0.05926
			Gelose Maize		
pure (A)	0.000 ± 0.156	0.0000 ± 0.0021	0.0162 ± 0.0031	0.0000 ± 0.0042	0.0000
sample 1 (B)	0.942 ± 0.070	0.0126 ± 0.0009	0.1318 ± 0.0087	0.3592 ± 0.0030	0.03128
sample 2 (C)	1.942 ± 0.112	0.0262 ± 0.0015	0.1999 ± 0.0076	0.6000 ± 0.0010	0.04905
sample 3 (D)	2.415 ± 0.218	0.0327 ± 0.0030	0.2380 ± 0.0059	0.6873 ± 0.0075	0.05373
sample 4 (E)	3.375 ± 0.062	0.0462 ± 0.0009	0.3441 ± 0.0049	1.0000 ± 0.0083	0.07274
			Hi-Maize		
pure (A)	0.000 ± 0.009	0.0000 ± 0.0001	0.0198 ± 0.0086	0.0000 ± 0.0081	0.0000
sample 1 (B)	1.413 ± 0.112	0.0189 ± 0.0015	0.1520 ± 0.0127	0.3745 ± 0.0015	0.04772
sample 2 (C)	2.126 ± 0.040	0.0287 ± 0.0005	0.2160 ± 0.0083	0.5406 ± 0.00781	0.05727
sample 3 (D)	3.000 ± 0.021	0.0409 ± 0.0003	0.2907 ± 0.0175	0.7930 ± 0.0015	0.07119
sample 4 (E)	4.148 ± 0.103	0.0572 ± 0.0015	0.3827 ± 0.0151	1.0000 ± 0.0063	0.08999
			Wheat		
pure (A)	0.000 ± 0.427	0.0000 ± 0.0057	0.0005 ± 0.0112	−0.0001 ± 0.0076	0.0000
sample 1 (B)	1.084 ± 0.029	0.0145 ± 0.0004	0.0834 ± 0.0031	0.3200 ± 0.0125	0.02423
sample 2 (C)	1.852 ± 0.007	0.0250 ± 0.0001	0.1620 ± 0.0009	0.5843 ± 0.0081	0.04315
sample 3 (D)	2.755 ± 0.009	0.0375 ± 0.0013	0.2341 ± 0.0310	0.7653 ± 0.0066	0.06173
sample 4 (E)	3.170 ± 0.086	0.0433 ± 0.0012	0.2928 ± 0.0156	1.0001 ± 0.0135	0.07323

[a] Determined using the titration method described in the Materials and Methods Section. [b] Determined using the integrated area measurements described in the text for the C=O and C=C stretch Raman bands in the 1600−1760 cm^{-1} region to the those of the C−C stretch bands in the 810−975 cm^{-1} region (represented by I_{1641}/I_{941}). [c] Determined using the CLS method described in the text to find the intensities for the C=O and C=C stretch Raman bands in the 1600−1760 cm^{-1} region to the those of the C−C stretch bands in the 810−975 cm^{-1} region (represented by I_{1641}/I_{941}). [d] Determined using the NMR intensity ratios of the integrated areas of the three maleate bands divided by the integrated area of the equatorial proton band of the native starch (see text).

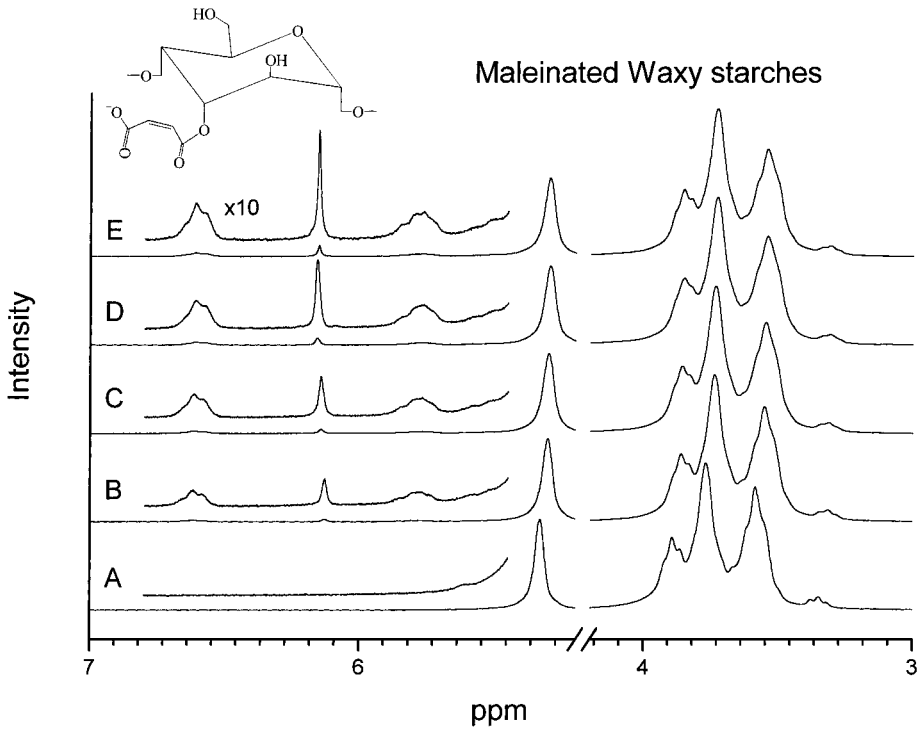

Figure 1. NMR spectra of maleinated waxy maize starch samples with varying degrees of substitution: pure starch ~0% maleinated (A), ~4% maleinated (B), ~6% maleinated (C), ~8% maleinated (D), and ~10% maleinated (E).

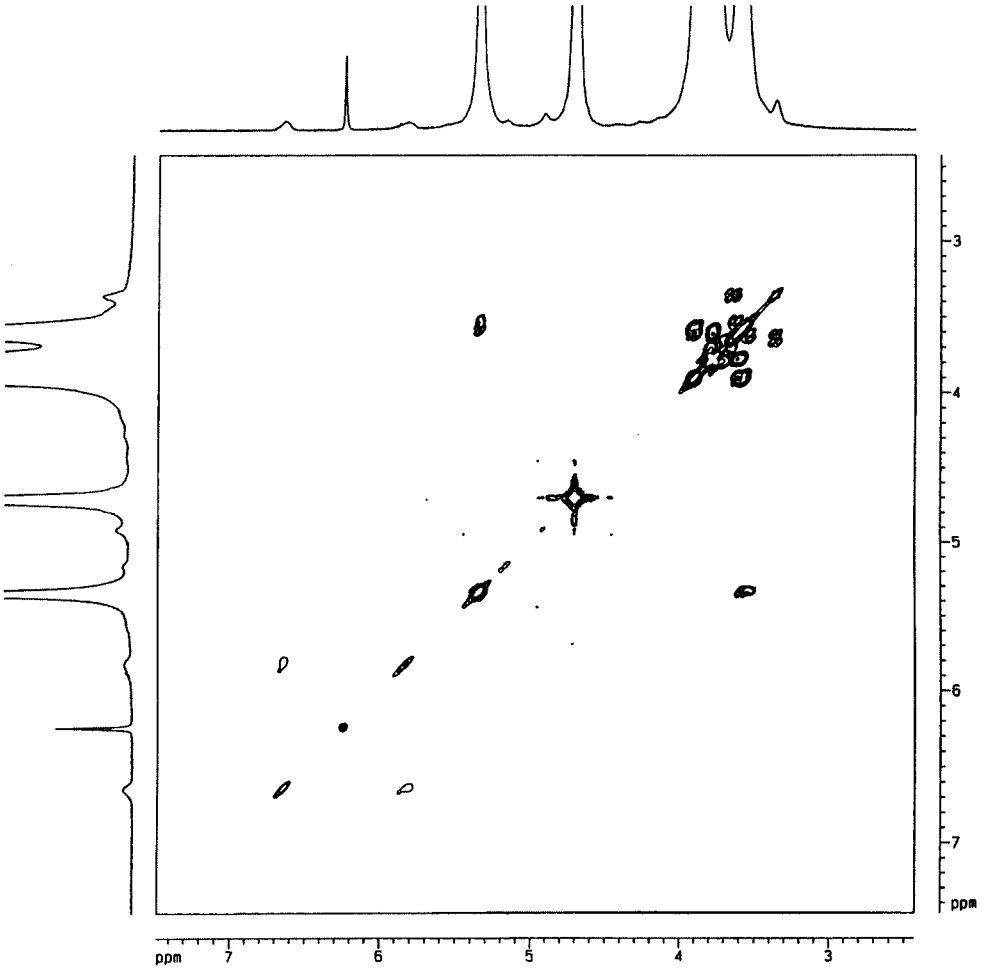

Figure 2. Hydrogen−hydrogen NMR correlation (COSY) spectrum of a maleinated waxy maize starch sample.

for 12 h. The excess alkali was back-titrated with a standard 0.1000 N hydrochloric acid solution and re-titrated 2 h later to account for any further alkali that may have leached from the starch.

Method for Collection and Analysis of NMR Spectra. Each starch sample was dispersed in D_2O and heated with a heat gun until the formation of a clear viscous solution was observed. All samples were measured on a Bruker 350 DPX spectrometer using a 90° pulse with presaturation of the water resonance at 4.5 ppm. 200 scans were acquired for each sample. The NMR data were then transferred to a PC computer after phasing, and peak integration was then performed.

Method for Collection and Analysis of Raman Spectra. The Raman spectra of the maleinated starch samples were obtained using a Fourier transform Raman (FT-Raman) spectrometer (Bio-Rad, Cambridge, MA) using 1064 nm excitation. Starch samples were placed in glass capillary tubes and the FT-Raman spectra were collected in a 180° backscattering geometry using about 100 mW of 1064 nm laser light and a collection time of about 7 min with a resolution of 8 cm^{-1}. As the Raman bands of silica from the capillary tube were very small and did not overlap with any Raman bands of interest, the Raman spectra were used directly for the area and least squares calculations. This helped to reduce any errors that would be introduced from spectral subtractions.

RESULTS AND DISCUSSION

The titrimetric method is commonly used to determine the degree of maleination in chemically modified starch. Maleinated starch samples are hydrolyzed by a standard alkali solution, and the amount of maleic acid modification in the sample is equivalent to the amount of alkali neutralized in the hydrolysis process which is found by back-titrating the resulting alkali solution with a standard hydrochloric acid solution. The level of modification of the starch samples is usually given as weight percent of maleate or degree of maleate substitution (D. S.). Table 1 gives the results obtained for the maize and wheat maleinated starch samples examined in our study.

The titrimetric method has several disadvantages. First, it is a destructive method that requires about 1.0 g of starch sample. Second, it is a time-consuming method that requires the cost and disposal of hazardous chemicals. Third, it is not practical for development as a technique for quality assessment in a process control situation. We have applied NMR and Raman spectroscopies to determine the degree of substitution of maleate in maleinated starches in order to develop nondestructive methods that require less time to obtain results than the titrimetric methods currently used in industry. NMR and Raman spectroscopic methods developed to measure the D. S. also use less hazardous chemicals and have the potential for development in a quality control situation.

The NMR spectra of pure waxy maize starch and four maleinated waxy maize starches (with differing levels of substitution) are shown in Figure 1. The NMR bands in the 3.3 to 4.2 ppm range are due to the anhydroglucose units of starch; the water band is at 4.6 ppm, and the anhydroglucose equatorial band is at 5.3 ppm. The maleinated starches exhibit new bands because of the maleate substitution: the bands around 5.5 and 6.6

Table 2. Parameters for Linear Regression Analysis of the Calibration Curves for the Raman Intensity Ratio or NMR Intensity Ratio versus the Degree of Substitution (D. S.) of Maleate in Modified Starches[a]

Parameters for Calibration Curve of D. S. of Maleate vs Raman Intensity Ratio Determined Using the Area Method Described in the Text

sample	*B*	*A*	*r*	LOD[b]
waxy maize	6.8741	−0.0006	0.9978	0.0047
regular maize	6.3897	0.0123	0.9991	0.0015
Gelose maize	6.7951	0.0261	0.9945	0.0065
Hi-Maize	6.3460	0.0272	0.9987	0.0038
wheat	6.6537	−0.0054	0.9973	0.0044

Parameters for Calibration Curve of D. S. of Maleate vs Raman Intensity Ratio Determined Using the CLS Method Described in the Text

sample	*B*	*A*	*r*	LOD[b]
waxy maize	18.436	0.0253	0.9957	0.0047
regular maize	27.067	0.0530	0.9955	0.0047
Gelose maize	20.828	0.0393	0.9944	0.0066
Hi-Maize	17.725	0.0249	0.9964	0.0064
wheat	22.134	0.0019	0.9943	0.0065

Parameters for Calibration Curve of D. S. of Maleate vs NMR Intensity Ratio Determined As Described in the Text

sample	*B*	*A*	*r*	LOD[b]
waxy maize	1.4119	0.0103	0.9951	0.0056
regular maize	1.5259	0.0043	0.9992	0.0016
Gelose maize	1.2148	0.0160	0.9965	0.0043
Hi-Maize	1.1124	0.0260	0.9995	0.0020
wheat	1.6667	0.0005	0.9989	0.0022

[a] $Y = A + BX$ where Y = ratio of the Raman intensity of the 1600−1760 cm^{-1} region C═O and C═C stretch bands and 941 cm^{-1} region C−C stretch bands determined using either the area method or the CLS method (see text) or the NMR intensity ratio described in the text, B = slope of the linear regression curve, A = intercept of the linear regression curve, X = degree of substiutution of maleate. $N = 5$ for all samples. [b] LOD = limit of detection as defined in the text.

ppm are due to the two nonequivalent protons of the monosubstituted maleate, and the band at 6.1 ppm is due to the two equivalent protons on the C═C of the di-substituted maleate. These assignments are consistent with the hydrogen−hydrogen correlation (COSY) NMR spectrum shown in Figure 2 which shows that the 5.5 and 6.6 ppm protons are correlated with each other and the 6.1 ppm protons do not interact with any other protons. The equatorial proton band at 5.3 ppm served as an internal standard, and the total integrated area of the three maleate bands (5.5, 6.1, and 6.6 ppm) was found to determine the D. S. The ratio of the integrated area of the three maleate bands divided by the integrated area of the equatorial proton band is shown in Table 1 and the linear regression results of the calibration curves are given in Table 2.

The Raman spectra for pure waxy, regular, Gelose 50, and Hi-Maize, and their maleinated starches are shown in Figure 3. The Raman bands in the 900 cm^{-1} region are mostly due to C−C stretches and the bands in the 2800−3000 cm^{-1} region are due to C−H stretches. The 1657 cm^{-1} Raman band with varying intensities in the native maize starch spectra appears to correlate with the amount of amylose present in the maize starches (*19*). Three new Raman bands appear in the Raman spectra of the maleinated starch spectra of Figure 3 and seem to correlate with the D. S. of the maleate modification. The new 1641, 1657, and 3046 cm^{-1} bands are

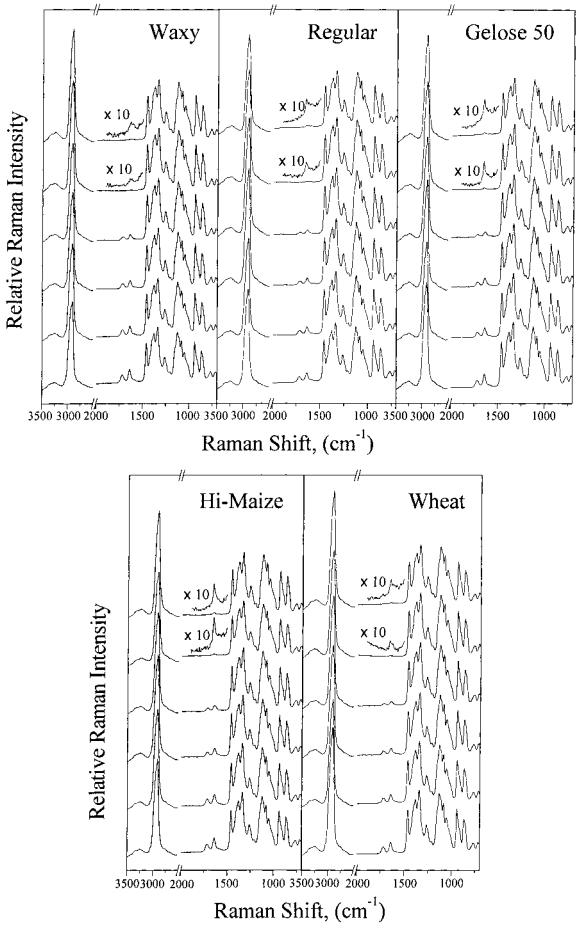

Figure 3. FT-Raman spectra of waxy, regular, Gelose 50, and Hi-Maize maize, and wheat starches with varying degrees of substitution of maleate and a control (native) starch sample. The maleate C═O and C═C signature Raman bands in the 1600−1760 cm^{-1} region grow substantially in intensity as the degree of substitution increases. The small 1657 cm^{-1} Raman band due to amylose provides a small constant background in the maleate signature Raman bands (see text).

likely due to the nominal C═O stretch, C═C stretch, and O−H stretch vibrational modes.

We have used two different methods to analyze the Raman spectra to investigate the correlation of the Raman band intensities of the C═O and C═C stretch Raman bands to the degree of substitution (D. S.) of maleate in chemically modified starches: an area method and a CLS method. The first analysis method uses integrated Raman band intensities of the region between 1600 and 1760 cm^{-1} to obtain the total Raman intensity of the C═O and C═C stretch Raman bands. The 810 to 975 cm^{-1} region associated with the C−C stretch Raman bands of the parent starch was also integrated for use as an internal standard between different spectra. The plot of the ratio of the integrated areas of the C═O and C═C stretch Raman bands in the 1600−1760 cm^{-1} region to the integrated areas of the C−C stretch bands in the 810−975 cm^{-1} region (represented by I_{1641}/I_{941}) versus the D. S. of maleate determined from the titrimetric method is shown in Figure 4. The linear regression of the plot given in Figure 4 is summarized in Table 2. The small amylose and amylopectin Raman bands at 1657 and 1637 cm^{-1}, respectively (*19*), are overlapped with the C═O and C═C stretch Raman bands, but do not affect the linearity of the correlation to the amount of maleate substitution.

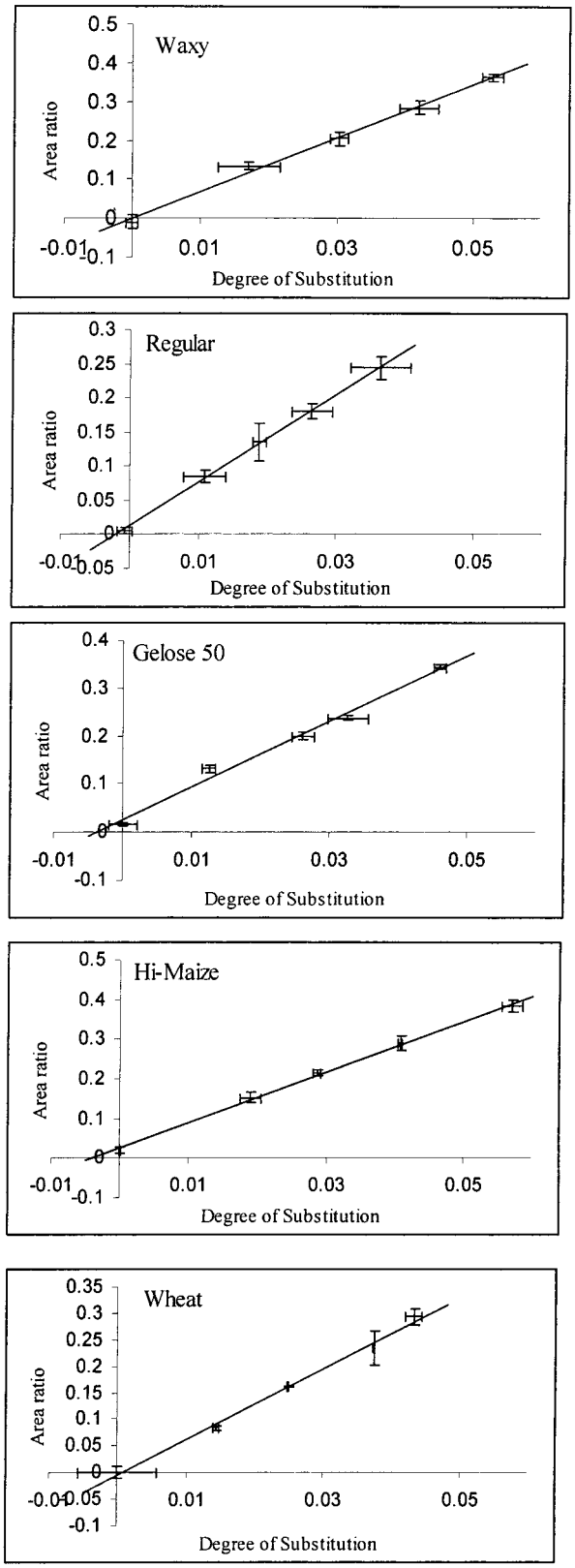

Figure 4. Plots of the ratio of the integrated areas of the C=O and C=C stretch Raman bands in the 1600−1760 cm^{-1} region to the integrated areas of the C−C stretch bands in the 810−975 cm^{-1} region (represented by I_{1641}/I_{941}) versus the degree of substitution of maleate determined from the titrimetric method. The error bars of the data points represent ± one standard deviation of the Raman (*y*-axis) and titration (*x*-axis) measurements. The linear regression parameters for these plots are summarized in Table 2.

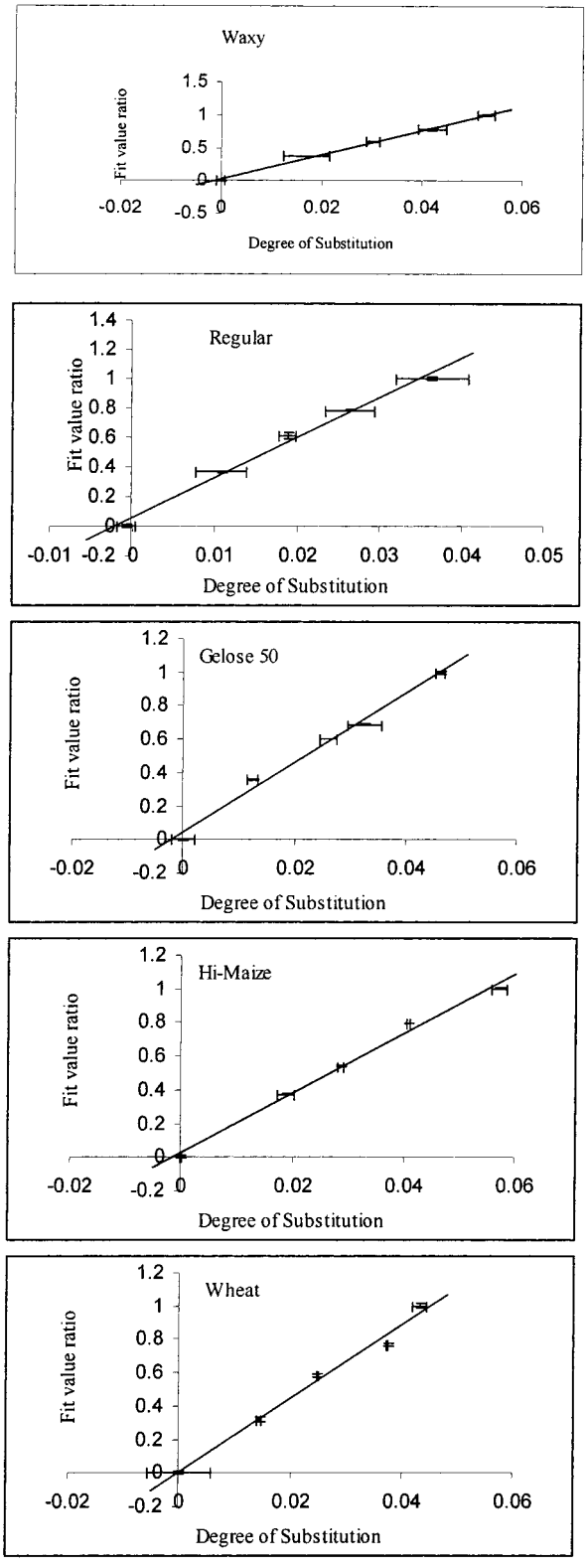

Figure 5. Plots of the ratio of the intensities (determined by the CLS method described in the text) of the C=O and C=C stretch Raman bands in the 1600−1760 cm^{-1} region to those of the C−C stretch bands in the 810−975 cm^{-1} region (represented by I_{1641}/I_{941}) versus the degree of substitution of maleate determined from the titrimetric method. The error bars of the data points represent ± one standard deviation of the Raman (*y*-axis) and titration (*x*-axis) measurements. The linear regression parameters for the plots are summarized in Table 2.

The small background due to the amylose (1657 cm^{-1}) and amylopectin (1637 cm^{-1}) Raman bands gives rise

Table 3. Determination of the Degree of Substitution of Maleate for Waxy Maize Samples with Unknown Degrees of Substitution of Maleate Using the Raman and NMR Spectra and the Calibration Curves Given in Table 2. Comparison to Separate Results Using the Standard Wet Chemistry Technique Are Used to Find the Relative Error (RE) of the Spectroscopic Methods

Determined using Raman spectra and calibration curves

sample	D. S. of maleate[a]	area[b] ratio I_{1641}/I_{941}	D. S. determined by area calibration curve	RE = (D. S. − area D. S.)/D. S.
waxy maize				
sample U1	0.0361 ± 0.0042	0.2583 ± 0.0064	0.0377	−0.0429
sample U2	0.0167 ± 0.0033	0.1106 ± 0.0021	0.0162	0.0308
sample U3	0.0096 ± 0.0017	0.0621 ± 0.0009	0.0091	0.0493
sample U4	0.0147 ± 0.0024	0.0963 ± 0.0067	0.0141	0.0404
sample U5	0.0198 ± 0.0043	0.1323 ± 0.0067	0.0193	0.0225

Determined using Raman spectra and Raman CLS calibration curves

sample	D. S. of maleate[a]	CLS[c] ratio I_{1641}/I_{941}	D. S. determined by CLS calibration curve	RE = (D. S. − CLS D. S.)/D. S.
waxy maize				
sample U1	0.0361 ± 0.0042	0.7045 ± 0.0011	0.0368	−0.0201
sample U2	0.0167 ± 0.0033	0.3235 ± 0.0067	0.0162	0.0305
sample U3	0.0096 ± 0.0017	0.2111 ± 0.0052	0.0101	−0.0504
sample U4	0.0147 ± 0.0024	0.3101 ± 0.0086	0.0155	−0.0517
sample U5	0.0198 ± 0.0043	0.4015 ± 0.0098	0.0204	−0.0316

Determined using Raman spectra and calibration curves

sample	D. S. of maleate[a]	NMR[d] ratio $I_{maleate}/I_{starch}$	D. S. determined by NMR calibration curve	RE = (D. S. − NMR D. S.)/D. S.
waxy maize				
sample U1	0.0361 ± 0.0042	0.0587	0.0343	0.0500
sample U2	0.0167 ± 0.0033	0.0347	0.0173	−0.0368
sample U3	0.0096 ± 0.0017	0.0244	0.0100	−0.0392
sample U4	0.0147 ± 0.0024	0.0301	0.0140	0.0448
sample U5	0.0198 ± 0.0043	0.0388	0.0202	−0.0202

[a] Determined using the titration method described in the Materials and Methods Section. [b] Determined using the integrated area measurements described in the text for the C═O and C═C stretch Raman bands in the 1600−1760 cm^{-1} region to the those of the C−C stretch bands in the 810−975 cm^{-1} region (represented by I_{1641}/I_{941}). [c] Determined using the CLS method described in the text to find the intensities for the C═O and C═C stretch Raman bands in the 1600−1760 cm^{-1} region to those of the C−C stretch bands in the 810−975 cm^{-1} region (represented by I_{1641}/I_{941}). [d] Determined using the NMR intensity ratios of the integrated areas of the three maleate bands divided by the integrated area of the equatorial proton band of the native starch (see text).

to somewhat different *y*-intercepts with the slope changing with amylose content in the linear regression plots (Figure 4) and parameters (Table 2). This indicates that one needs different Raman calibration curves for starches with noticeably different amylose contents to achieve the best results for the determination of the D. S. in maleinated starch samples with unknown amounts of modification.

A second method of analysis to develop Raman calibration curves was performed using classical least-squares (CLS) treatment of the Raman spectra. Four components were incorporated into the reference set for the calculations. These components included the ensemble averages of the control (0% D. S.) and the highest D. S. samples. In addition, a set of constant values and a ramp were included to account for the uneven background contribution to the sample spectra from fluorescence. The calculations were carried out for the 1600−1760 cm^{-1} region of the C═O and C═C stretch bands and the 810−975 cm^{-1} region of the internal standard C−C stretch bands. The least squares values of each component was found for the samples from the CLS calculations. These values should lie between 0 and 1 and represent the fraction of each component that contributed to the sample spectrum. We assume that the components are mutually independent of each other. The sample spectrum can be reconstructed by linear combinations of the four components and their least-squares values. The ratio of least-squares value for the starch reference to that of the control should correct for any variations during data acquisition. Ideally, the resulting values obtained from the CLS calculation should give 0 for the pure starch and 1 for the highest D. S. sample examined for each type of starch. Tables 1 and 2 list the results of the CLS calculations and their linear regression results of the least-squares values versus the degree of substitution of maleate (also plotted in Figure 5).

Inspection of Table 2 reveals that both the NMR and Raman methods display a very high degree of linearity for their marker band intensities with the degree of maleate substitution, and their correlation coefficients are >0.99 for all of the types of starches examined in our study. One of the commonly accepted definitions of the limit of detection (LOD) is three times the standard deviation of the *y*-intercept divided by the sensitivity of the calibration curve and is used to represent the detectability of an analytical method. The LODs for each of the Raman and NMR calibration curves are given in Table 2 and their values lie in the range of 0.0015 and 0.0066. Both the NMR and Raman methods have similar sensitivities for the detection of the degree of substitution of maleate in starch samples. The NMR and Raman calibration curves can be used to determine the degree of maleate substitution in unknown starch samples. We determined the degree of maleate substitution for a set of waxy maize samples with unknown amounts of substitution using the calibration curves for the two Raman based methods and the NMR method for validation purposes (Table 3). The actual degree of

substitution was determined using the standard wet chemistry technique for the unknown waxy maize samples and these results are also shown in Table 3. All of the Raman, NMR, and wet chemistry measurements were done in triplicate. Inspection of Table 3 shows that the relative error (RE) between the standard wet chemistry technique value and those determined from the Raman and NMR calibration curves of Table 2 and the appropriate spectroscopic measurements are on the order of 0.05 or less. This indicates that both the Raman and NMR spectroscopic measurements and their calibration curves can be used with confidence for determination of the degree of substitution of maleate for chemically modified starches with an accuracy similar to that of the standard wet chemistry method commonly used.

Both the NMR and Raman methods for the determination of the degree of maleate substitution in starch presented here are nondestructive measurements that allow the starch sample to be recovered for use in other characterization studies. More importantly, the NMR and Raman methods provide much faster determination of the degree of maleate substitution than the currently used wet chemistry titrimetric method. The NMR and Raman methods also use no hazardous chemicals and generate no hazardous waste. Raman spectroscopy can be used to make measurements of maleinated starch samples in a variety of environments (such as in aqueous or nonaqueous solutions or dry solids) and therefore requires almost no sample preparation. Thus, both the NMR and Raman spectroscopic methods presented here offer substantial improvements for the fast, accurate, and easy-to-obtain determination of the degree of substitution of maleate in starch. The NMR and Raman techniques can potentially be further developed for noninvasive in-line remote sensing to provide quality control analysis in an industrial environment, something that is not practical for the titrimetric method.

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