

Influencing Factors for Determining the Crystallinity of Native Cellulose by X-ray Diffraction

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Full width at half maximum (FWHM) of diffraction peaks of native cellulose is larger than 1° . The diffraction peaks of the crystalline phase largely coincide with those of the amorphous phase of cellulose, leading to the low resolution. Therefore, when calculating the crystallinity of natural cellulose by fitting the peaks, only relying on the single evaluation factor of Rwp (R-weighted Pattern) may lead to great randomness of calculation results. Due to the special crystal structure of natural cellulose and the characteristics of crystallinity determination by peak separation method, the XRD Rietveld fitting method is adopted to determine the crystallinity of native cellulose. Through limiting the convergence conditions of fitting functions, we firstly discuss the effects of peak shape functions, scanning range, and the positions of amorphous peaks on crystallinity determination, which helps to reduce the randomness of XRD in solving crystallinity of cellulose and improve the precision of calculation. Then, three evaluation indexes (Rwp, FWHM, and RSD) are used to evaluate the rationality of crystallinity calculation results. Moreover, the reproducibility and precision of the crystallinity of three kinds of natural cellulose are tested under the optimized conditions. At the same time, the optimized fitting method is adopted to calculate the content of cellulose I and II, which can be used to guide the selection of alkali concentration and alkali treatment time in the spinning process of native cellulose.

Keywords XRD, Rietveld peak fitting, cellulose crystallinity, reproducibility, rationality

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Introduction

As a kind of homomorphic polycrystalline macromolecular compound, cellulose is composed of many microcrystallines of about 10 nm (crystal phase), which are distributed in the matrix of a completely random molecular chain.¹ Native cellulose consists of cellulose I, a small amount of cellulose II and the amorphous phase.² The amorphous phase is mainly composed of lignin, hemicellulose, pectin, *etc.*³ Crystallinity is defined as the fraction of the crystalline portion in the testing sample, and its measurement methods mainly include XRD,⁴ NMR,^{5,6} FT-IR,^{7,8} and Raman⁹ (Table S1, Supporting Information). Among them, XRD is the most important test method because of its provides information on definite physical properties.¹⁰⁻¹⁵ The crystallinity tested by XRD does not require a standard substance, chemical composition or other structural information, and can realize the separation of overlapping diffraction peaks. XRD-based crystallinity calculation methods are as follows: empirical method,¹⁶ Hermans-Weidinger method,¹⁷ Jayme-Knolle method,¹⁸ and Rietveld method.¹⁹ During the crystallinity calculation with Rietveld peak fitting method, besides crystallinity, cell parameters,²⁰ grain size,²¹ full width at half maximum (FWHM),²² and other parameters can also be obtained. Therefore, XRD is widely used

in the study of the crystal structure of native cellulose.

The grain size of cellulose I is generally less than 10 nm, and the FWHM of the diffraction peak is larger than 1° .²³ It is well known that the diffraction peaks of the three crystalline planes (101, $10\bar{1}$, and 002) are distributed in the range of $15 - 23^\circ$, which coincides with the distribution range of diffraction peaks of the amorphous phase. Therefore, it is not easy to distinguish the crystalline phase from the amorphous phase when calculating the crystallinity by fitting the fractional peak. In general, Rwp has been the only index has been used to evaluate the fitting effect of Rietveld peak separation method, but it is only applicable to the case that the diffraction peaks of the crystalline phase are significantly separated from the amorphous phase. If the diffraction peaks of the crystalline phase largely coincide with the amorphous phase, Rwp cannot differentiate the crystalline phase from the amorphous phase. The minor changes in testing conditions may lead to a large randomness of testing results. Even worse, typical characteristic diffraction peaks will be omitted after fitting, which makes such fitting results not conform to the physical characteristics of crystallography. For example, Park *et al.*²⁴ only obtained the crystalline plane (10 $\bar{1}$) of cellulose II in the process of peak fitting.

In order to obtain satisfactory results with the Rietveld refinement method, it is necessary to correctly describe the diffraction peak shapes. The FWHM of diffraction peaks and the symmetry of peak shape all vary with 2θ . FWHM is mainly related to the crystal structure, grain size, and the preferred

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orientation, whereas the peak shape is relevant to the performance with the monochromator and the geometric characteristics of the instrument collimation system. For making different peak shape functions suitable for full spectrum, the selection of functions plays a very key role. Here, according to the characteristics of peak separation method and by controlling the convergence condition of the fitting function, we have discussed the effects of peak shape function, scanning range and position selection of the amorphous peak on the measurement of crystallinity for the first time, which helps to reduce the randomness of XRD in solving crystallinity of cellulose and improve the precision of calculation. It also provides a good reference for the researchers engaged in the calculation of cellulose crystallinity.

Experimental

Materials and methods

The natural cellulose used in our experiments was cypress pulp for spinning (cellulose content >99.5%, Jilin Chemical Fiber Co., Ltd.). A Bruker D8 FOCUS Powder X-ray Diffractometer (Germany Bruker Corporation) was used for the crystallinity determination. TOPAS P3 (Bruker, Germany) was used as the software for resolving overlapping bands.

Sample processing and testing

Samples were dried at 90°C for 2 h and then completely crushed with scissors for testing. XRD patterns were obtained with the Bruker D8 FOCUS Powder X-ray Diffractometer (Bruker, Germany) under the following test conditions: Cu target ($K\alpha = 0.15418$ nm), tube current of 40 mA, tube voltage of 40 kV, DS slit of 0.5°, SS slit of 0.5°, RS slit of 0.1 mm, step scan mode, scan rate of 0.02°, and scan step width of 2 s/step. TOPAS P3 (Bruker, Germany) software was used in the peak analysis.

Selection of peak shape functions

In this paper, the crystallinity calculated by Rietveld refinement method is mainly performed with four functions, and the specific form of the functions is shown in Table S2 (Supporting Information).

Effect of amorphous peak position

Based on the calculation formulas of the empirical method and scattering spectra of completely amorphous polymer (lignin), 18 – 21° was selected as the fitting starting angle of amorphous peak and fitted by Rietveld refinement method. Then the fitting results were compared with those calculated by the empirical method,¹⁶ which is shown in Eq. (1):

$$\text{CrI} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100\%, \quad (1)$$

where CrI is crystallinity index; I_{002} is the intensity of the diffraction peak at $2\theta = 22.8^\circ$; I_{am} is the intensity of the scattering peak at $2\theta = 18.5^\circ$.

Effect of the scanning range

The corresponding diffraction angles of the four crystal planes (101, 10 $\bar{1}$, 002, 040) of the natural cellulose were 14.98, 16.7, 20.64, 22.8 and 34.49°, respectively, so the test ranges were set to be 3 – 40, 5 – 40, 7 – 40, 10 – 40°, respectively.

Selection of fitting methods

According to the characteristics of Rietveld bands separation

Table 1 Effect of peak shape functions

Function	PV	PVII	Mod-pV	Mod-PVII	Empirical value
CrI, %	97.00	98.75	99.99	99.87	90.20
Rwp	15.15	15.06	14.99	14.80	—

fitting method, automatic peak search and appointed peak position were adopted for peak fitting.

Effect of FWHM convergence condition

According to the characteristics of each crystalline plane in the two crystals of cellulose I and cellulose II, FWHM convergence conditions of crystalline peaks and amorphous peaks are restricted, respectively. The FWHM convergence conditions of crystal peaks based on the three crystalline planes of wood cellulose with higher crystallinity, and those of amorphous peaks refers to the FWHM of the scattering peaks of pure lignin. Experimental conditions of three tests are shown in Table S3 (Supporting Information).

Reproducibility experiments

Three kinds of cellulose, wood, cotton and bamboo, were tested by the above optimized conditions, and their Rwp, FWHM, and RSD ($n = 5$) were calculated, respectively.

Results and Discussion

Peak shape function

In this study, four indexes were used to evaluate the crystallinity calculation of different peak shape function fitting. Firstly, the calculation of Rwp (R-weighted Pattern). Because Rietveld spectrum refinement uses special functions to fit the experimental spectrum, the better the fitting effect, the smaller value of Rwp.²⁵ Secondly, comparison of fitting and empirical calculation results. In recent years, there has been a great deal of literature about the CrI calculation of cellulose by empirical formula. Thirdly, the study and determination of the shape and FWHM of the amorphous peak. Fourthly, the study on the repeatability of calculation results. The results calculated with various peak shape functions in Table S2 (Supporting Information) are presented in Table 1.

From Table 1, Rwp values fitted by the four functions are all about 15, indicating that the four functions show no significant difference in the fitting error. But compared with the results calculated by the empirical method, the PV function calculated in the four peak shape functions has a better correlation. Therefore, we choose the PV function to calculate cellulose crystallinity.

Position selection of amorphous peaks

The amorphous part of natural cellulose is mainly composed of disordered cellulose, hemicellulose, lignin, pectin, and other components, so the composition is relatively complex. As shown in Fig. 1, the FWHM values of four crystalline planes of native cellulose are large (all larger than 1°), and the diffraction peaks of the crystalline phase and the amorphous phase coincide significantly. The above factors make it difficult to confirm the position of the amorphous peaks. Therefore, it is very important to select the appropriate amorphous peak position for fitting. We selected 17 – 21° as the highest point of the amorphous peak for fitting, which was based on the scattering spectrum of the

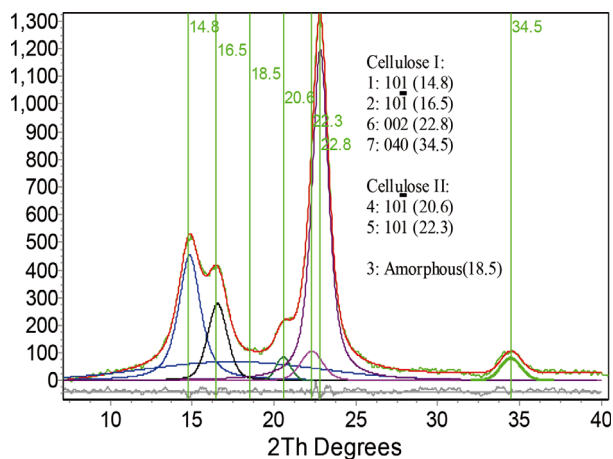


Fig. 1 XRD patterns of native cellulose.

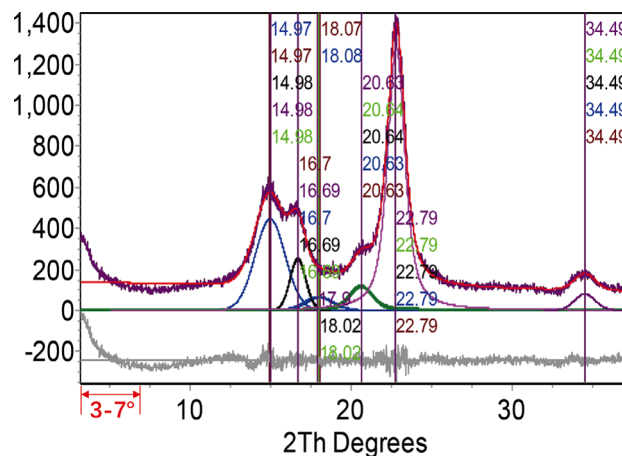


Fig. 2 Fitting results of different amorphous peaks (17 - 19.5°).

Table 2 Effect of amorphous peak position

Non-crystalline position	17	18	18.5	19	19.5	20	21
CrI, %	96.40	96.81	96.17	96.19	96.78	98.65	84.00
Rwp	15.14	15.15	15.14	15.14	15.15	15.22	15.15

same kind of amorphous polymer lignin,²⁴ and the calculation results are presented in Table 2.

As illustrated in Table 2, Rwp values obtained by fitting amorphous peaks at different locations are basically the same. However, the value of Rwp can only evaluate the coincidence between the experimental spectrum and the fitting spectrum. As can be seen from CrI value, which shows high reproducibility when the fitting initial angle is 17 - 19.5°, the crystal peaks and the amorphous peaks under different fitting conditions are basically overlapped (see Fig. 2). Furthermore, amorphous peaks are all at the location of 18°, which coincides with the results of the empirical method. When the fitting angle exceeds 19.5°, the crystallization calculation results fluctuate greatly. Therefore, 17 - 19.5° was selected as the fitting initial angle of amorphous peak, by which the obtained CrI value shows high reproducibility and RSD reaches 0.32%.

Scanning range

As seen in Fig. 2, at a low angle (3 - 7°), different background lines can be caused by the incoherent scattering of lights and direct irradiation of X-ray.²¹ For samples with high crystallinity, the phenomenon of high diffraction intensity will be covered up. The poor crystallinity and small grain size (<10 nm) of natural cellulose obviously, caused the widening of diffraction peak and low peak strength, thus making the background value higher at the low angle. The calculation results obtained according to the experimental conditions in the above (effect of the scanning range) are provided in Table 3.

Table 3 shows that the value of Rwp decreased gradually while the initial scanning angle increased. When the initial angle increases to 7°, RWP decreases to 6.71, and as the initial angle continues to increase, RWP remains basically unchanged. This is mainly because the significant effects of incoherent scattering of lights and direct irradiation of X-ray at a low angle (3 - 7°) can induce high background lines, causing obvious fitting errors and interference with the calculation of RWP and

Table 3 Effect of scanning range

Initial angle/°	3	5	7	9	11
CrI, %	96.99	84.23	82.66	88.99	91.53
Rwp	15.15	7.40	6.71	6.59	6.51
FWHM/°	1.80	4.76	7.60	3.46	1.68

FWHM. With the increasing of the initial scanning angle, FWHM of the amorphous peak firstly decreases and then increases. When the initial angle is 7°, FWHM is larger than 7°, which is consistent with FWHM of scattering spectra of amorphous lignin. Therefore, when the initial angle is 7° and the scanning range is 7 - 40°, the baseline drift will gradually disappear and the Rwp value will become smaller and smaller, which is consistent with results reported by Rojith *et al.*⁷

Fitting methods

The symmetry of the diffraction peak at 22.79° is poor in Fig. 1, because native cellulose contains a small amount of cellulose II. We know cellulose II has two kinds of crystalline planes (10 $\bar{1}$, 101), which respectively correspond to the 2 θ values of 20.6 and 22.3°. Therefore, when using the appointed peak position fitting, crystalline planes of 20.1 and 21.9° are ascribed to cellulose II. According to the experimental conditions (*Selection of fitting methods*), the fitting results are shown in Fig. 3.

As demonstrated in Fig. 3a, only one diffraction peak is fitted out of cellulose II at 20.58° by seeking peak automatically. There are two kinds of crystal planes (10 $\bar{1}$ and 101) in cellulose II, and the corresponding angles of 2 θ are 20.6 and 22.3° respectively, while those of 002 in cellulose I are 22.8°. When the content of cellulose I is high, the 101 crystal plane (22.3°) of cellulose II can be completely concealed. Therefore, only one crystal plane (10 $\bar{1}$) is fitted by automatic peak search method. For example, Park *et al.*²⁴ only obtained the crystal plane (10 $\bar{1}$) in cellulose II during the fitting process. However, when the appointed peak fitting is adopted (Fig. 3b), two crystal planes (10 $\bar{1}$, 101) of cellulose II are mandatorily added, and according to the setting conditions, the shape of the diffraction peak can be fitted optimally when the function performs fitting. In this way, the omission of unobvious diffraction peaks because of the coincidence of the crystal plane will be prevented. Due to the lack of diffraction peak (101) in the automatic peak-finding

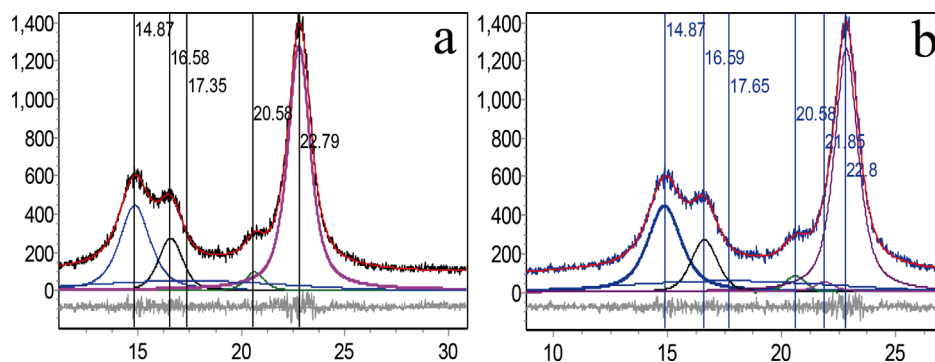


Fig. 3 Influences of fitting ways (a) automatic peak search; (b) appointed peak position.

Table 4 Comparison of the fitting results obtained under different convergence conditions

No.	1			2			3		
	FWHM/ $^{\circ}$	CrI, %	$A_{10\bar{1}}/A_{101}$	FWHM/ $^{\circ}$	CrI, %	$A_{10\bar{1}}/A_{101}$	FWHM/ $^{\circ}$	CrI, %	$A_{10\bar{1}}/A_{101}$
Cellulose									
Wood	8.00	88.67	0.11	0.98	99.27	1.15	10.77	84.06	0.97
Cotton	12.40	77.48	0.02	11.34	66.23	1.82	11.33	67.23	1.20
Bamboo	11.42	84.31	0.01	12.01	66.00	1.06	12.01	66.74	1.30

Note: $A_{10\bar{1}}/A_{101}$ represents the area ratio of the two crystalline planes ($10\bar{1}$, 101) of cellulose II.

fitting results, which does not conform to the physical characteristics of crystallization, the calculation results of crystallinity are less than those of the appointed peak position fitting. In this paper, the fitting model has been adopted with the appointed peak position, so that each diffraction peak of natural cellulose can be fitted out. Furthermore, not only the crystallinity can be calculated accurately, but also the respective content of the two cellulose phases (I and II) can be obtained through corresponding peak area. The above calculation results are used for guiding the selection of alkali concentration and alkali treatment time in the spinning process of natural cellulose.

Effect of FWHM convergence conditions

According to the experimental conditions in Table S3 (Supporting Information), Rietveld fitting calculation is carried out for the crystallinity of wood, bamboo and cotton, and the results are summarized in Table 4. As demonstrated in Table 4, different convergence conditions have a great influence on the results of FWHM, CrI, and $A_{10\bar{1}}/A_{101}$. When the convergence conditions of the amorphous peak is FWHM $> 8^{\circ}$, the $A_{10\bar{1}}/A_{101}$ values of the three cellulose II are less than 0.1.

Figure 4 shows the refinement results of the pure cellulose II spectrum, and we can see that $A_{10\bar{1}}/A_{101}$ of two crystalline planes ($10\bar{1}$, 101) is about 1.4. When the convergence condition of the amorphous peak is set as FWHM $> 8^{\circ}$, the fitting results are obviously inconsistent with actual results. When the convergence condition of the crystalline peak is selected as FWHM $< 2^{\circ}$, FWHM of the amorphous peaks of wood cellulose is 0.98° , and the crystallinity is 99.27%. The above two fitting results significantly deviate from the actual values. However, when the convergence conditions 1 and 2 are met simultaneously, the FWHM average value of three kinds of cellulose is 11° , which is basically consistent with that obtained when the amorphous lignin is used as the standard substance. The $A_{10\bar{1}}/A_{101}$ of the three types of cellulose II are 0.97 – 1.3, which is in accordance

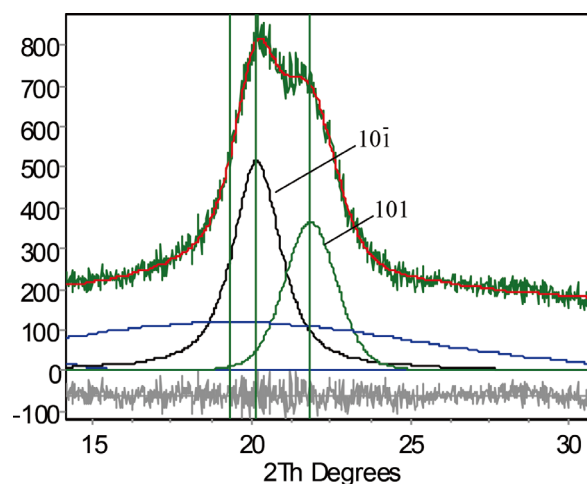


Fig. 4 XRD fitting spectra of cellulose II.

with the ratio of pure cellulose II. The slight difference of $A_{10\bar{1}}/A_{101}$ is mainly ascribed to the grain refinement of different cellulose. Therefore, if the convergence conditions of amorphous peaks and crystalline peaks are limited at the same time, more reliable FWHM and $A_{10\bar{1}}/A_{101}$ of amorphous peaks can be achieved.

Reproducibility test

According to the above optimization conditions were set as follows: fitting function, PV; amorphous peak position, 18° ; scanning range, 7 – 40° ; convergence conditions of amorphous peak, FWHM $> 8^{\circ}$; and convergence conditions of crystalline peak, FWHM $< 2^{\circ}$. The test was repeated 5 times and referred to as “reproducibility experiments”. The results are shown in

Table 5 Reproducibility testing results.

Cellulose	Wood	Cotton	Bamboo
CrI, %	83.14	66.17	67.02
Rwp	6.61	6.34	6.12
FWHM ^o	8.80	9.20	8.90
RSD	3.13	4.41	3.72

Table 5. The average crystallinity of the three kinds of cellulose was 83.14, 66.17, and 67.02, respectively. Rwp was around 6, and FWHM was larger than 8°, which is consistent with the FWHM of lignin. RSD were 3.13, 4.41, and 3.72%, respectively, and the reproducibility of experimental data was very good.

Conclusions

In summary, the fit function, the position of the amorphous peak, the scanning range, the fitting method, and the reproducibility and stability of FWHM convergence condition to the crystallinity calculation were investigated. After optimization, the parameters were set as follows: the fitting function, PV; the position of amorphous peak, 18°; scanning range, 7 – 40°; fitting model, appointed peak position fitting; convergence condition of amorphous peak, FWHM > 8°; and convergence condition of crystalline peak, FWHM < 2°. Based on the optimized conditions, we have tested the crystallinity of three kinds of native cellulose, and the three evaluation indexes (Rwp, FWHM, and RSD) were in conformity related requirements. The developed method for crystallinity determination of cellulose can be used to guide the selection of alkali concentration and alkali treatment time in the spinning process of native cellulose.

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Supporting Information

Table S1 Comparison of calculating methods for cellulose crystallinity; Table S2 Different profile functions; Table S3 FWHM convergence conditions. This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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