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A CP/MAS ^{13}C NMR investigation of molecular ordering in celluloses

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Abstract

The individual states of order of cellulose found within *Valonia*, *Cladophora*, *Halocynthia*, cotton and wood cellulose were quantified by non-linear least-squares fitting of the ^{13}C NMR spectra. The results from the spectral fittings indicated that a cellulose form giving signals at δ 104.5 (C-1 region) and δ 88.1–88.5 (C-4 region) was present in all the investigated samples. Partial signal suppression by spin–lattice relaxation supported the findings from the spectral fittings. The spectral behavior of this cellulose form indicates it is a less-ordered or a para-crystalline 'in core' structure with a somewhat larger mobility than the crystalline cellulose Iα and Iβ allomorphs. © 1997 Elsevier Science Ltd.

Keywords: Cellulose; CP/MAS ^{13}C NMR; Supermolecular structure; Order; Allomorphs

1. Introduction

Natural cellulose fibers have a molecular architecture with a high degree of individuality, depending on their biological origin. There are nevertheless several organizational features that are generally found in all such fibers. Based on results from CP/MAS ^{13}C NMR spectroscopy on several native cellulose materials, VanderHart and Atalla concluded [1–3] that the crystalline domains in native celluloses are composites of two phases, cellulose Iα and cellulose Iβ. Cellulose Iα is the dominant form in bacterial and algal celluloses, whereas cellulose Iβ is the dominant form in higher plants and tunicate celluloses [1–8].

In samples of *Valonia* (algae) and *Halocynthia* (tunicate) celluloses, with large lateral and highly ordered domains, the relative amounts of cellulose Iα and Iβ have previously been determined by non-linear least-squares fitting of a superposition of Lorentzian lines to appropriate parts of the ^{13}C NMR spectra [6,8]. For both celluloses it was necessary to involve an additional line, in the crystalline C-4 region not assignable to cellulose Iα or Iβ, to obtain a satisfactory description of the spectra. It was suggested that this line, although not completely accounted for, originated from crystalline [6] or partially distorted domains of cellulose or that it had its origin in an oversimplification of the spectra due to the use of Lorentzian line-shapes [8].

Since the *Valonia* and *Halocynthia* celluloses represent extremes of natural celluloses with regard to both the sizes of the ordered domains and their allomorph composition (i.e. the former is Iα-rich and the latter is Iβ-rich) it is interesting to note the presence of a common and hitherto unassigned line in the NMR spectra of both types of cellulose. This

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raises the question of whether there are in general domains in the cellulose fibrils characterized by states of order other than traditionally expected, e.g. amorphous cellulose and crystalline cellulose Iα and cellulose Iβ. The unambiguous assignment of this extra line to a true cellulose form, however, requires the investigation of a large set of cellulose samples with dissimilar solid-state compositions.

In this paper, cellulose samples from different sources have been examined by subjecting appropriate regions of their CP/MAS ^{13}C NMR spectra to non-linear least-squares fitting. In the spectra of all the samples, the presence of a signal at δ 88.1–88.5 (in the crystalline C-4 spectral region) could be substantiated and, in the case of the investigated plant cellulose, this signal constituted a major contribution. This C-4 signal is assigned to domains within the fibrils of somewhat higher mobility (lower order) than those found in the cellulose Iα and cellulose Iβ domains.

2. Results and discussion

Samples.—Although cellulose is a simple homopolysaccharide, it can exhibit a multitude of solid-state forms depending on its origin and on the isolation methods used. To investigate the general presence of an additional cellulose form in the crystalline C-4 spectral region of a cellulose I type of materials [6,8], we chose a set consisting of highly ordered *Valonia*, *Cladophora*, and *Halocynthia* cellulose, less-ordered cotton cellulose and wood cellulose of low order isolated from birch kraft pulp. The cellulose samples were all subjected to a mild acid hydrolysis to remove other contaminating polysaccharides and thus facilitate the decomposition of the NMR signals from cellulose. The CP/MAS ^{13}C NMR spectra of the celluloses are shown in Fig. 1. It is apparent that the solid-state structure differs widely between the samples. The most pronounced tendency through the series is the deterioration of spectral details from highly ordered to less-ordered cellulose. This is most clearly visible in the C-1 (δ 101–107) and C-4 (δ 80–91) regions of the spectra.

Signal quantitativity.—When cellulose signal clusters are decomposed into contributing lines by a non-linear least-squares fitting of NMR spectra, the question of the quantitativity of the CP/MAS ^{13}C NMR spectra arises. The ^{13}C signal intensities in a spectrum obtained by a CP/MAS pulse sequence depend on the rotating-frame time constants $T_{1\rho}^{H}$, $T_{1\rho}^{C}$,

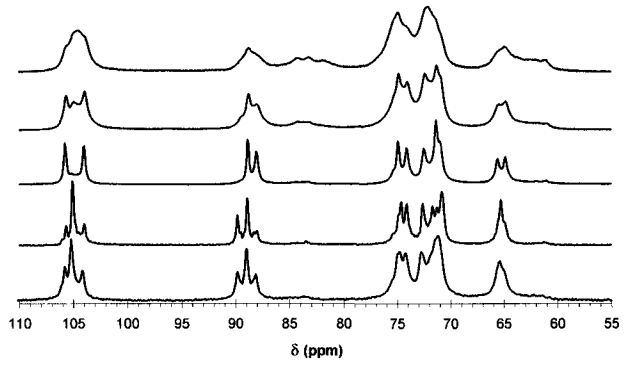

Fig. 1. CP/MAS ^{13}C NMR spectra of the different celluloses used. From bottom to top the order is: *Valonia* cellulose, *Cladophora* cellulose, *Halocynthia* cellulose, cotton linters, and bleached birch pulp.

and T_{CH} [9,10]. It is by no means evident that cellulose molecules which can exists in several different states of order and mobility exhibit the same rotating-frame relaxation behavior during cross-polarization. This may result in the suppression of signals from some of the components of the sample and, hence, in a non-quantitative spectrum. To investigate whether or not this is so in the case of cellulose, two samples of cotton linters, one wet (40–60% water by weight) and one dry (5–10% water by weight) were mixed with polyethylene with a known quantitative relation (determined gravimetrically) between cellulose and polyethylene. In order not to affect the influence of amorphous components, cotton linters were chosen for the quantitativity experiments.

The spectrum of the mixed sample of polyethylene and wet cotton linters is shown in Fig. 2. The results of the cross-wise quantification of polyethylene and wet and dry cotton linter samples are presented in Table 1. Both samples exhibit close agreement with the expected value, indicating that 95–100% of the

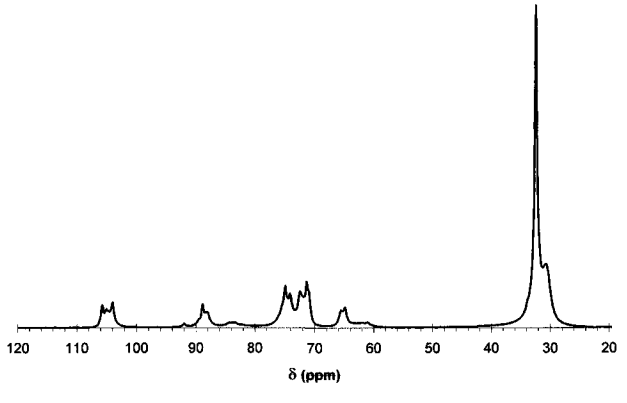

Fig. 2. CP/MAS ^{13}C NMR spectrum of wet cotton linters mixed with polyethylene.

Table 1
The results of the spectral integration of the wet and dry samples of cotton linters mixed with high-molecular-weight polyethylene. The CP/MAS NMR ratio is determined by dividing the integrated signal intensity in the polyethylene region with the integrated signal intensity in the cellulose region. The values in parentheses are the standard deviation

Sample	CP/MAS NMR ratio	Gravimetrical ratio
Wet cotton linters and polyethylene	1.14 (0.05)	1.12
Dry cotton linters and polyethylene	1.05 (0.07)	1.12

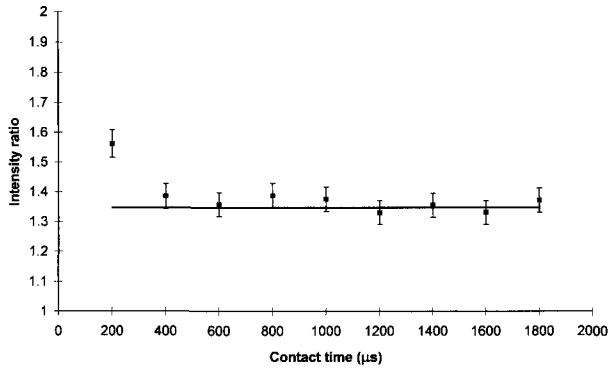

Fig. 3. Cross-wise quantification of wet cotton linters and polyethylene at several contact times. The squares give the intensity ratios obtained by integration of the polyethylene and cellulose spectral regions, the error-bars give the ±3% error limits, and the horizontal line gives the expected intensity ratio determined gravimetrically (1.35).

carbohydrate content is detectable in the CP/MAS ^{13}C NMR spectra. Fig. 3 shows the results obtained when the experiment was repeated with a sample at several contact times. Taken together, this constitutes evidence against any substantial discrimination of signals from the different states of order in cellulose, since the chemical and structural differences between cellulose and polyethylene mean that the probability of the signal suppression being equal is very small.

Spectral fitting.—In addition to minimizing the squared residual during the fitting procedure, using the model given in Eqs. (1)–(4), two further acceptance criteria were used to discriminate between acceptable and non-acceptable fitting results (see Experimental section). Attempts were made to decompose the C-1 and C-4 regions of cotton cellulose. This could not be achieved using Lorentzian lines only. However, if a mixed model containing both Lorentzian and Gaussian lines was used, a satisfactory description of the experimental spectra was obtained and both acceptance criteria were met. The results of the non-linear least-squares fitting of the C-1 and C-4 regions of the cotton cellulose are shown in Fig. 4a and b. The assignments, widths, relative intensities, and line types are given in Tables 2 and 3. For the number and types of functions used, the overall description of the experimental spectra is satisfactory. In the C-1 region (Fig. 2a), six lines have been used. In addition to the lines originating from the cellulose Iα, cellulose Iβ, and cellulose II, two large and wide lines at δ 104.5 and 102.4 are visible. The assignments of these two wide lines are not clear, since it is not obvious whether the less-ordered components in the C-1 region are split into two signals, or whether they produce an asymmetric signal that is decomposed into two symmetric lines by the fitting procedure. This could be the case, since the mathematical model used contains only symmetric lines. The assignment of the small line at δ 105.9 to cellulose II relies on the chemical shift value and on a recent estimation, by principal component analysis of CP/MAS ^{13}C NMR spectra, of low amounts of cellulose II in cotton linters [11].

The signals originating from the different states of order in the C-4 region of the spectrum of cotton cellulose (Fig. 4b) are distributed over a wider shift range than the signals in the C-1 region, and this makes a more detailed analysis possible. In the high-

Table 2
The results of a non-linear least-squares fitting of the C-1 region of the CP/MAS ^{13}C NMR spectrum of cotton cellulose. Values in parentheses are standard errors. Only errors larger than 0.1 ppm are quoted for the chemical shift values

Assignment	δ (ppm)	FWHH (Hz)	Intensity (%)	Line type
Cellulose II	105.9	30 (6)	1.8 (0.6)	Lorentz
Iβ	105.6	31 (1)	16.8 (0.6)	Lorentz
Iα	104.9	39 (3)	7.1 (0.8)	Lorentz
Less-ordered	104.5	156 (6)	45.5 (2.2)	Gauss
Iβ	103.9	33 (1)	16.2 (0.4)	Lorentz
Less-ordered	102.4 (0.29)	413 (31)	12.6 (1.1)	Gauss

Table 3
The results of a non-linear least-squares fitting of the C-4 region of the CP/MAS ^{13}C NMR spectrum of cotton cellulose. Values in parentheses are standard errors. Only errors larger than 0.1 ppm are quoted for the chemical shift values

Assignment	δ (ppm)	FWHH (Hz)	Intensity (%)	Line type
Iα	89.5	29 (3)	2.2 (0.3)	Lorentz
I($\alpha+\beta$)	88.7	30 (1)	14.9 (0.6)	Lorentz
Para-crystalline	88.4	153 (5)	33.1 (1.3)	Gauss
Iβ	87.9	53 (2)	14.7 (1.1)	Lorentz
Amorphous	84.9 (0.35)	479 (39)	24.1 (2.0)	Gauss
Fibril surface	84.1	80 (10)	4.9 (0.7)	Gauss
Fibril surface	83.2	60 (12)	2.9 (1.0)	Gauss
Hemicellulose and cellulose oligomers	82.3 (0.41)	135 (56)	3.1 (1.6)	Gauss

field end of the region, the overlap from the C-2, C-3, and C-5 signals is modeled as a single line with only its low-field tail visible in this portion of the spectrum. The amorphous cellulose is visible as a very wide line covering almost the entire C-4 region. No cellulose II signals could be distinguished in this region, possibly due to the low amount (judging from the results obtained from the C-1 region). In addition to the Iα and Iβ signals in the crystalline region (δ 86–91), one large and wide line centered at δ 88.4 were visible.

In the amorphous region (δ 80–86), two distinct signals of approximately equal area are visible, and these are believed to be signals originating from the crystallographically inequivalent cellulose fibril surfaces [12]. The results from the non-linear least-squares fitting of the C-1 and C-4 regions quantify consistently, i.e. the same degree of crystallinity is predicted from both regions (when fibril surface signal intensities are taken into account) and is in agreement with what is expected from the mixed or composite crystal model.

The results from cotton cellulose and similar quantifications obtained from the C-4 region of wood cellulose (birch), *Halocynthia* cellulose (tunicate) [8], *Cladophora* cellulose (algae), and *Valonia* cellulose (algae) [6] are compiled in Table 4. In the cases of the highly ordered celluloses (originating from *Halocynthia*, *Cladophora*, and *Valonia*) fitting was performed with Lorentzian lines only, and the amorphous region (δ 80–86) was fitted with a single line only due to its low intensity. The reported data for *Valonia* were reproduced from the work by Yamamoto and Horii [6]. (The chemical shifts reported by Yamamoto and Horii differ from those obtained by our laboratory by an offset of 0.4 ppm. This is probably due to differences in the procedure used for calibrating the chemical shift scale.) In both algal celluloses, one small line is distinguishable at δ 89.5. Due to its low relative intensity, about 3%, no attempt was made to assign this line. The featureless character of the spectrum of wood cellulose is typical for a cellulose of low order. The lack of spectral detail makes an exhaustive analysis impossible. Typically, this results in an inability to distinguish the individual from the cellulose Iα and Iβ allomorphs. Only the largest cellulose I($\alpha+\beta$) signal is distinguishable.

Table 4
Quantifications made by non-linear least-squares fitting of the C-4 region in the ^{13}C NMR spectra. All values are relative intensities in percent, and values given in parenthesis are standard errors

Cellulose source	Cellulose Iα	Cellulose Iβ	Para-crystalline cellulose	Surface cellulose	Amorphous cellulose	Hemicellulose and cellulose oligomers	Unassigned signal intensity
Wood	9.1 (0.7)		31.1 (0.7)	6.0 (0.8)	53.7 (0.9)		–
Cotton	4.2 (0.5)	27.6 (1.8)	33.1 (1.3)	7.8 (1.2)	24.1 (2.0)	3.1 (1.6)	–
Halocynthia	9.6 (1.8)	61.2 (11.6)	12.5 (3.5)		16.7 (1.6)		–
Cladophora	51.7 (1.1)	29.4 (1.6)	4.5 (0.8)		11.4 (1.0)		3.1 (0.4)
Valonia [a]	55.9	25.1	7.0		7.0		5.0

[a] Data taken from ref. [6].

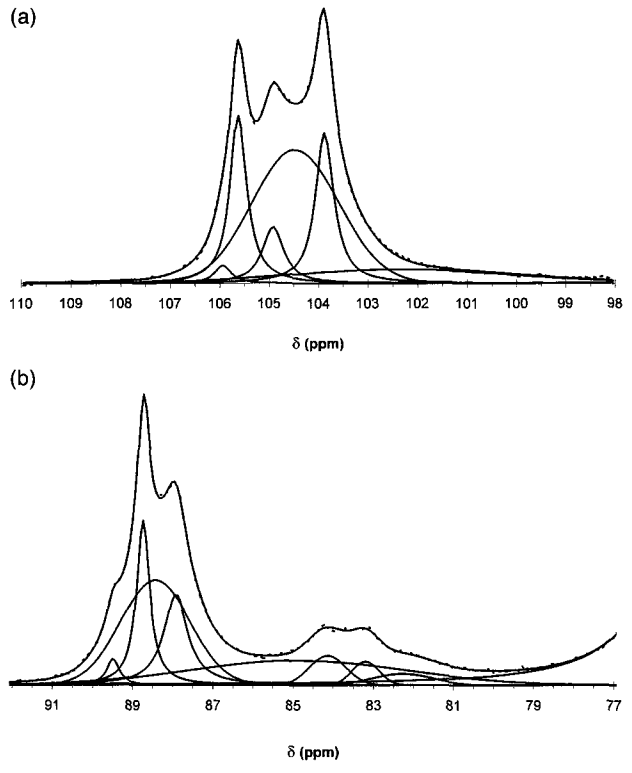

Fig. 4. Result of the fitting of (a) the C-1 region and (b) the C-4 region of a CP/MAS ^{13}C NMR spectrum from cotton cellulose. The chemical shift, relative intensity, linewidth, and type of the individual lines are compiled in Table 2Table 3, respectively. The experimental spectrum is shown as a dashed line. The individual fitted lines and their superposition are shown as solid lines. The dashed line of the experimental spectrum is of limited visibility due to the superimposed fitted spectrum.

Partial signal suppression.—To test further the presence of an additional signal from cellulose in the crystalline C-4 region, the hypothesis was put forward that, since the well-known signals from cellulose Iα and Iβ in the C-4 region originate from highly ordered cellulose, the unexpected signal found by spectral fitting (δ 88.4) originated from some less-ordered (more mobile) state of cellulose. If this were the case, it might be possible to suppress, or at least partially suppress, this signal due to a difference in spin–lattice relaxation time (T_1). To test this hypothesis, an inversion recovery pulse sequence [13] was used with a single delay time. Normally this pulse sequence [13] is used with several delay times. This gives a set of spectra with the magnitude of a signal decay towards zero as a function of delay time. The rate of the signal decay depends on the molecular mobility at the atoms giving rise to the signal. Higher mobility results in a more rapid decay. This means that it may be possible to choose a single delay time in such a way that most of the ^{13}C signal intensity from the more mobile (less-ordered) components is quenched, while most of the ^{13}C signal intensity from the less-mobile (more ordered) components is retained. The reason for not performing a complete T_1 measurement (with several delay times) was the extremely long measurement time needed for such an experiment

The results from this measurement verified the presence of the para-crystalline signal. For the delay time chosen, the relative signal intensities of the crystalline components were retained, the surface and amorphous cellulose signals were completely quenched, and the intensity of the para-crystalline signal was reduced to 23% and 3%, respectively, of its unsuppressed value (relative to the signal intensities of the crystalline cellulose) in cotton linters and cotton cellulose. As a further indication of the significance of the signal at δ 88.4, Paci et al. [14] have recently reported signals originating from less-ordered cellulose in the crystalline C-4 spectral domain using a pulse sequence differentiating between sample components of different proton T_2 relaxation times.

Supermolecular structure.—The partial signal suppression supports the findings from the spectral fittings of the cellulose spectra without any relaxation delay, and this constitutes evidence against the signal observed at δ 88.1–88.5 being an artifact due to the arbitrary nature of the non-linear least-squares fitting procedure. Furthermore, the apparently shorter spin–lattice relaxation time of the δ 88.1–88.5 signal suggests that this signal originates from a cellulose form with a state of order or mobility intermediate between the crystalline cellulose Iα and Iβ allomorphs on the one hand and the cellulose found on the fibril surfaces and amorphous cellulose on the other hand. The chemical shift of δ 88.1–88.5 that places the signal in the crystalline C-4 region and its relative abundance in the plant celluloses investigated indicates that this cellulose form is situated in the core of the fibrils. Our suggestion is thus that the observed cellulose form corresponds to a less-ordered or para-crystalline core structure [15] with a somewhat larger mobility than that of the crystalline cellulose Iα and Iβ allomorphs.

3. Conclusion

An investigation of the individual states of order in celluloses by non-linear least-squares spectral fitting

and partial signal suppression indicates the presence of a cellulose form giving NMR signals at δ 104.5 (C-1) and δ 88.1–88.5 (C-4). The spectral behavior of the cellulose form indicates that it is a less-ordered (more mobile) or para-crystalline 'in-core' structure with a somewhat larger mobility than that of the crystalline cellulose Iα and Iβ allomorphs.

4. Experimental

Sample preparation.—*Halocynthia* cellulose was prepared from commercially available tunicate (*Halocynthia sp.*) [8]. *Cladophora* cellulose was isolated from *Cladophora sp.* naturally grown in the Baltic sea and prepared according to a procedure due to Gardner et al. [16], followed by hydrolysis in aq 2.5 M HCl at 100 °C for 30 min. The cotton cellulose was prepared by hydrolysis of commercially available cotton linters in aq 2.5 M HCl at 100 °C for 20 min. The wood cellulose was prepared by hydrolysis of bleached birch kraft pulp in aq 2.5 M HCl at 100 °C for 4 h. The xylan content in this sample was 2%.

The samples used to estimate the quantitativity of the NMR spectra of cellulose was a mixture of high-molecular-weight (MW $> 3 \times 10^6$) polyethylene and cotton linters. The cellulose and polyethylene contents in the sample were determined gravimetrically and by integration of spectra.

NMR spectroscopy.—All spectra were recorded using wet samples (water content 40–60% by weight) except for one of the mixed polyethylene and cotton linter samples where dry (water content 5–10% by weight) cotton linters was used. The CP/MAS ^{13}C NMR spectra were recorded on a Bruker AMX-300 instrument (at ambient temperature) operating at 75.43 MHz. A double air-bearing probe and zirconium oxide rotor was used. The MAS rate was 5000 Hz. Acquisition was performed with a CP pulse sequence using a 3.5-ms proton 90° pulse, a 800-ms contact pulse, and a 2.5-s delay between repetitions. Glycine was used for the Hartmann–Hahn matching procedure and as an external standard for the calibration of the chemical shift scale relative to tetramethylsilane [$(CH_3)_4Si$]. The data point of maximum intensity in the glycine carbonyl line was assigned a chemical shift of 176.03 ppm.

The partial signal suppression experiment was performed by conducting two measurements, one with no delay and one with a 200-s delay, using a pulse sequence that utilizes cross-polarization in the initial build-up phase of the magnetization [13]. The experiment was carried out with both cotton linters and cotton cellulose.

Fitting of spectra.—The mathematical model used for the non-linear least-squares fitting [17] of the CP/MAS ^{13}C NMR spectra includes two line types, Lorentzian and Gaussian. The reason for going beyond a model of a single line type was an attempt to extend the applicability of the method to spectra of less-ordered celluloses. The two functions [corresponding to the same value of i in Eq. (1)] are given the same central frequency and the same full width at half height (FWHH).

$$S(\omega) = \sum_{i=1}^{n} w_i^G G_i(\omega) + w_i^L L_i(\omega) \quad (1)$$

$$G_i(\omega) = \frac{1}{\sigma_i\sqrt{2\pi}} \exp\left(\frac{-(\omega-\omega_i)^2}{2\sigma_i^2}\right) \quad (2)$$

$$L_i(\omega) = \frac{1}{\pi} \frac{2\tau_i}{1+4(\omega-\omega_i)^2\tau_i^2} \quad (3)$$

$$\sigma = \frac{1}{\tau\sqrt{\ln(256)}} \quad (4)$$

In this model, each signal is characterized by four parameters: the chemical shift (ω_i), the spread (τ_i or σ_i), and the weights of the Gaussian and the Lorentzian function (w_i^G and w_i^L, respectively). For the expressions used, the FWHH is given by $1/\tau_i$.

To be able to handle the large degree of arbitrariness introduced by using up to four parameters per line, restrictions on an acceptable fit in addition to minimizing the sum of squared residuals were imposed by demanding agreement with the mixed or composite crystal model due to VanderHart and Atalla [1,2,6] and the quantitative agreement of relative amount estimates obtained from different spectral regions (i.e. the C-1 and C-4 regions of a spectrum). The algorithm used was to select a model (specify n and a set of initial parameter values in Eqs. (1)–(4) and select which parameters to adjust during fitting to spectra) for each of the selected spectral regions. Once the fitting procedure has converged, the results are accepted or rejected according to the general quality of the fit and the two criteria: (i) agreement with the mixed or composite crystal model and (ii) consistent quantifications from different spectral regions.

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