

How Crystalline is My Cellulose Specimen? Probing the Limits of X-ray Diffraction

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Cellulose serves as a skeleton for many of the useful products upon which we rely on each day. When we want to learn about a skeleton, it makes sense to think about X-ray methods. The same can be said when it comes to learning about the crystallinity of cellulose. Over the past six decades, the Segal X-ray diffraction (XRD) method has been popular for judging the percent crystallinity of powder samples. However, XRD patterns for ideal cellulose crystals can be easily simulated, and limitations of the Segal and other methods become obvious. Calculated patterns for model 100% crystalline powder particles are predicted to be less crystalline by the Segal method. Except for the Rietveld method, current approaches do not account for particle orientation or different shapes of crystallites. The Rietveld method has so many variables that it can easily overfit the data. The take-away message is that routine XRD examination is important for showing sample characteristics, but fractional crystallinity values are affected by constraints related to simplifications required for the analysis.

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How to Determine Cellulose Crystallinity from XRD Data – A Presentation by Dr. Al French

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Foundations of X-ray Diffraction for Evaluation of Cellulose

Diffraction crystallography, with a history of 110 years, has been the subject of 29 different Nobel Prize Awards to 48 individual Laureates (Nobel, n.d.). This makes clear that crystal structure study is on a firm foundation. However, we must distinguish between cellulose crystal structure and cellulose crystallinity. The crystal structures, including their x, y, and z atomic coordinates, for most of the several neat crystal forms were solved in the first decade of this century by *e.g.*, Langan *et al.* (2001) and Nishiyama *et al.* (2002). That seminal work, taking advantage of synchrotron X-ray beams and neutron beams from a nuclear reactor, provided at least a convincing and fully testable set of structures that have not been surpassed. Those studies relied on carefully selected and prepared samples that provide much more data than samples of materials of daily commerce such as wood pulp or cotton fibers. Even so, materials such as ramie fibers contributed to the historic understanding of the polymeric nature of cellulose and other polymers.

It is often said that cellulose has both crystalline regions and amorphous regions or phases. A two-phase model is almost certainly a simplification, but the aspiration is to be able to at least describe convincingly how much of each is present, along with the characteristics of the crystalline phase. Because of the reduced diffraction information

available from practical samples, especially when in the convenient powder form, this aspiration remains elusive, leaving cellulose crystallinity as a research frontier.

Powder Diffraction Studies

Numerous methods for studying cellulose crystallinity exist, but many are correlated to diffraction methods rather than an independent and direct study of the crystalline character of the sample. For that reason, researchers often turn to powder diffraction; some 30% of the papers in the *Cellulose* journal include a powder diffraction analysis. The reason for the popularity is that many different treatments can potentially change the sample from one crystal form to another or change the balance of crystalline and amorphous phases. Perhaps too much importance has been attributed to this balance (the degree of crystallinity) in determining the properties of cellulose, but the importance cannot be fully ascertained until we can be confident of our measurements of crystallinity.

The Segal *et al.* (1959) equation for cellulose crystallinity is some 63 years old, and according to Google Scholar, it has been cited some 6700 times. Citers include the paper on cellulose crystallinity methods by Park *et al.* (2010), which by 2018 was the most cited article in *Biotechnology for Biofuels and Bioproducts*. That work and a similar work by Thygesen *et al.* (2005) described the various ways to analyze the diffraction pattern for crystallinity. Besides the Segal peak height method, “amorphous subtraction,” “peak deconvolution,” and Rietveld methods are used.

A great way to evaluate the suitability of these methods is to examine the diffraction pattern for an ideal cellulose crystal. Any researcher can calculate powder diffraction patterns for ideal cellulose crystals. All that is needed is a computer, suitable software such as Mercury (Macrae *et al.* 2008), and a crystal information file, which is a convenient package of the details of the crystal structure such as atomic coordinates and the unit cell dimensions. Start the free Mercury software, load the crystal information file (.cif extension) provided with French (2014), and press the powder button on the default screen. This is as trivial as it gets in science. Of course, the pattern does not look like your cellulose pattern, but knowing what change is needed to make it look like your pattern is a valuable way to understand the crystalline material. Changes to the calculated pattern in Mercury and Rietveld programs are based on known physical phenomena that are incorporated in the calculations.

In the Mercury diffraction pattern window, press “Customize...”, “Pattern”, and change FWHM from the default 0.1° to 2°. Now the calculated pattern looks like a nice ramie or linen pattern; change to 3° and it might look more like a wood pulp-based cellulose nanocrystal. The crystallite size for a spherical crystal is related to the Full Width at Half Maximum (FWHM) through the Scherrer equation, so what has been done is to model the diffraction pattern for different sizes of crystallites. $\text{Size} = K \cdot \text{wavelength} / (\text{FWHM} \cdot \cos \theta)$, where K is a constant (often taken as 0.89), wavelength is the one used in the diffractometer or software to create the pattern, FWHM must be in radians (if you are doing a spreadsheet calculation), and θ is half of the 2θ value in your spreadsheet’s required units. Thus, you have modeled crystals of about 900 Å, 45 Å, and 30 Å. Here are some valuable lessons from this *in silico* experiment:

1. There are many different peaks in the ideal calculated pattern that blend together as the crystallites become smaller, contributing to the intensity that might mistakenly be considered “background” and subsequently removed, say by a 2-point method.
2. In the peak height methods, the indicator of the amount of amorphous material is the intensity at its minimum value near about 18.6° . In the 30 \AA crystal pattern, however, the I_{am} intensity is 2500 counts. Accordingly, the Segal CrI = $100 * (10000 - 2500) / 10000 = 75\%$, but there was no amorphous contribution in the input. The main reason that there was intensity at the “ I_{am} ” point is the fact that the small crystals gave broad peaks that overlap. This observation disqualifies the Segal and “amorphous subtraction” methods for any reading of absolute crystallinity, as all of these calculated patterns were for perfect crystals of various sizes. Of course, you might consider matter composed of small crystals to be less crystalline than matter composed of a few large crystals. That issue could be settled by the Scherrer equation calculations but is not consistent with the two-phase crystalline/amorphous model.
3. The 900 \AA pattern provides three other important observations. Miller indices are the technical term applied to the peak labels. The labels can be obtained by placing the mouse cursor on the peak. For example, the highest peak is shown to be the 2, 0, 0. You might know it as the 0, 0, 2 peak. We could both be correct, because these labels depend on the conventions used in the .cif. Different conventions have been used over the past 95 years or so. The *Cellulose* journal requires the convention that is used in the .cif for cellulose, namely with the c-axis unique, parallel to the molecular axis. This is the standard for most polymers but not for small molecules.

Note also that all the peaks in the patterns from the smaller crystals are overlapping with other ones, so that the smaller peaks are subsumed by larger ones. Consider the small one at 34.57° . In the high-resolution (900 \AA) pattern, this peak is rather insignificant, but it is the one chosen to represent the cluster of neighboring peaks when the peaks on the smaller-crystal patterns are labeled. Normally the larger peaks are chosen to represent the cluster, but the 0,0,4 peak at 34.57° is significant because it converts through the Bragg equation to exactly one fourth of the unit cell length. Because the peaks at 14.9° and 16.7° often overlap for the smaller crystals, it is not appropriate to choose one. Instead, designate the peak as 1-10/110. The positions of the peaks change slightly with crystal size.

The 900 \AA pattern provides input on a terrible error in the literature. Segal’s paper shows that the I_{am} is the intensity at the minimum near 18.0° , where the intensity of the amorphous component was maximal in his experiments on ball-milled cotton. Far too many workers have misread this and chosen the 1-10/110 peak as the indicator of amorphous content. I repeat that the .cif has no information about amorphous content, and it should therefore be obvious that the 1-10/110 peak is just another peak coming from the crystal lattice. Please help stamp out this error of using a peak instead of the minimum intensity.

The Mercury software is useful, but I jokingly call it a starter drug, leading to the Rietveld method. One fault of Mercury is that it doesn’t handle your data but only gives patterns to compare with yours. Cautions are that it exports .cifs for polymers with extra atoms unless you check the “Asymmetric unit” box, and the intensity falls off from the

correct value at the end of the range when the FWHM (also called pwhm) causes peak overlap. Calculated patterns also show that the “peak deconvolution” method, as usually implemented, is deficient because only a few of the many peaks are used to model experimental patterns. That problem leaves only the Rietveld method standing as a potentially satisfactory method. Among its problems are the numerous additional variables in a Rietveld analysis needed to take care of known complications with cellulose samples. These known problems include anisotropic crystallite size (the crystals are typically longer along the molecular axis than they are perpendicular to it) and preferred orientation (the crystallites tend to lie in the plane of the sample holder rather than be randomly oriented, the assumption used in calculation of the ideal pattern. Preferred orientation is especially problematic with bacterial cellulose and with films made from nanocellulose. The small number of unique data in a powder pattern can easily be overfit with so many variables.

In any case, experimental XRD patterns by themselves are valuable visual proof that a sample and its purity are as described. For new materials, XRD patterns are especially important. Because the standard deviation in counting statistics (noise) is the square root of the intensity value, the diffraction patterns for cellulose should have a peak height of an absolute minimum of 2000 counts for the highest cellulose peak; 100,000 counts is surely enough. Artificial smoothing can induce its own problems, so just get good data. A modern detector on a diffractometer can collect 100,000 counts at the main peak over a range of 5° to 60° 2θ with steps of 0.04° in about a half hour. Technical issues that make big differences and should be described include whether the reflection or transmission mode was used, and fixed or variable slits.

A current issue is the “environmental background,” which is independent of the sample. A controversial approach to removing “air scatter” and other background is to collect data from a blank run and subtract it from the data run. Try it and see if it does not make your pattern look more like an ideal one, even if you have a “zero background” sample holder. Background and amorphous scattering should be separated to help get a good fix on the crystalline/amorphous ratio because the amorphous scattering and the background scattering compete for the same low intensity part of the diffraction pattern.

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