REFERENCE MATERIALS FOR THE STUDY OF POLYMORPHISM AND CRYSTALLINITY OF CELLULOSE

T. G. Fawcett, C. E. Crowder and S. Kabbekodu, International Centre for Diffraction Data

J. A. Kaduk, Poly Crystallography Inc.
This document was presented at PPXRD - Pharmaceutical Powder X-ray Diffraction Symposium

Sponsored by The International Centre for Diffraction Data

This presentation is provided by the International Centre for Diffraction Data in cooperation with the authors and presenters of the PPXRD symposia for the express purpose of educating the scientific community.

All copyrights for the presentation are retained by the original authors.

The ICDD has received permission from the authors to post this material on our website and make the material available for viewing. Usage is restricted for the purposes of education and scientific research.

PPXRD Website – www.icdd.com/ppxrd  ICDD Website - www.icdd.com
Cellulose Polymorphs

Jack Griffith, University of North Carolina at Chapel Hill (via Fox News)

A bundle of cellulose fibers around 253 million years old, recovered from a salt deposit 2,000 feet beneath the ground in New Mexico.

Oldest known biological material
Collaborators and Contributors

- Jim Kaduk, Poly Crystallography Inc
- Tom Blanton, Eastman Kodak Co.
- Ewa Bucher, International Paper Company
- Fangling Needham, ICDD
- Cam Hubbard, Oak Ridge National Laboratory
- Valeri Petkov, Central Michigan University
- Roman Shpenchanko, Moscow State University

- Bruker–AXS & Glasgow University (PolySnap), PANalytical (HighScore Plus), CrystalMaker Software LTD (CrystalMaker)
- Rigaku, Bruker–AXS, PANalytical, Argonne NL Light Source– instrument time and expertise
Data Collection and analysis

2002–2007
- 12 Pharmaceutical Tablets – Fangling Needham, ICDD clinics, Cam Hubbard, Oak Ridge National Lab, Jim Kaduk, Argonne Light Source
- 3 Natural Products
- 18 Wood Pulps, Cotton Linters – Eva Bucher, International Paper

2010–2011
- 21 Wood chips – Jim Kaduk, Poly Crystallography Inc
- 6 USP references – ICDD editors, Joel Reid and Suri Kabekkodu, ICDD grantees, Victor Petkov, Roman Shpanchenko
- 6 Substituted cellulososes – Tom Blanton, Eastman Kodak, Suri Kabekkodu, ICDD
Elucidation of the structures of cellulose 1 alpha, cellulose 1 beta and cellulose II. Ab-initio refinements constrained by XRD, ED, nmr and SEM data.

Structures applied to powder patterns and used to identify polymorphism in wood pulps and pharmaceuticals. Reported the pattern of amorphous cellulose.
Polymorphs of Cellulose

Form 1 beta shown

PDF-4+
Faber, Scardi Leone

Pepcid AC

These simulations are exported and used to model experimental data

Simulation of microcrystalline states of cellulose

References for Form I alpha, Form I beta and Form II
Macro to micro to nano

Cell walls

Plant cells

Cellulose fibrils in plant cell wall (TEM)

Fibril

Microfibril

0.5 µm

polymers of beta glucose

Glucose monomer

Cellulose chains

City University of New York, Brooklyn, Biology Department
Surface of a tissue
Bundled fibers

AFM of Cellulose I alpha

Microscopy
Macro to Micro

Simulation from images, oxygens circled

Baker, Helbert, Sugiyama and Miles
Biophysical Journal Volume 79 August 2000 1139–1145

Microfibrils
Cellulose 1 beta and alpha

- (200) Cellulose I beta
- (-110) Cellulose 1 alpha

P21 – 2 distinct staggered sheets

P1 – Alternating Conformers in dimer but one sheet (AB)

No intersheet bonding in either alpha or beta

(200) 3.866 A
(-110) 3.823 A
Stable form
Intersheet hydrogen bonding
2 chains (AA or BB) antiparallel
Large –OH disorders (10–30%)
Confidence In Reference Standards

**Cellulose 1 beta, 50 A**
Microcrystalline Cellulose

**Cellulose I alpha, 25 A**
Lignum Vitae

**Cellulose II, 40 A**
Mercerized Pulp

Amorphous Ref
Pulp ground for 6.5 hrs

**Cellulose II, 40 A**
Mercerized Pulp
46 Experimental cellulose specimens compared to 4 cellulose standards (50A)

Yellow = Cell 1b
Blue = amorphous
Red = Cellulose II
Green = Cell 1a
## Crystallinity in processed and ground cellulosics

<table>
<thead>
<tr>
<th>Material</th>
<th>Phase</th>
<th>% Crystallinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigma 0 hr</td>
<td>1b</td>
<td>0.892</td>
</tr>
<tr>
<td>Filter Paper</td>
<td>1b</td>
<td>0.818</td>
</tr>
<tr>
<td>USP Microcrystalline</td>
<td>1b</td>
<td>0.818</td>
</tr>
<tr>
<td>Paper Pulp A handsheet</td>
<td>1b</td>
<td>0.921</td>
</tr>
<tr>
<td>Paper Pulp C Handsheet</td>
<td>1b</td>
<td>0.92</td>
</tr>
<tr>
<td>Microcrystall Aldrich</td>
<td>1b</td>
<td>0.818</td>
</tr>
<tr>
<td>Sigmacell</td>
<td>1b</td>
<td>0.892</td>
</tr>
</tbody>
</table>

### Sigmacell

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase</th>
<th>% Crystallinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>Ground</td>
<td>Amorph 0.911</td>
</tr>
<tr>
<td>Mercerized sheet</td>
<td>Pretreated</td>
<td>Amorph 0.871</td>
</tr>
<tr>
<td>Sigma 6.5 hr</td>
<td>Ground</td>
<td>Amorph 0.925</td>
</tr>
<tr>
<td>Mercerized 10 min</td>
<td>Ground</td>
<td>Amorph 0.923</td>
</tr>
<tr>
<td>Sigmacell 2 hr</td>
<td>Ground</td>
<td>Amorph 0.974</td>
</tr>
<tr>
<td>Sigmacell 3 hr</td>
<td>Ground</td>
<td>Amorph 0.982</td>
</tr>
<tr>
<td>Mercerized 1 hr</td>
<td>Ground</td>
<td>Amorph 0.97</td>
</tr>
<tr>
<td>Sigma 13 hr</td>
<td>Ground</td>
<td>Amorph 0.959</td>
</tr>
<tr>
<td>Sigma 10 hr</td>
<td>Ground</td>
<td>Amorph 0.941</td>
</tr>
</tbody>
</table>
Macro clustering reflects polymorphism and crystallinity

Note the natural products (roots) are mixed with the wood pulps.
Some conclusions

- Structures of Cellulose 1a, 1b and II along with experimentally derived amorphous cellulose can be used as references for polymorph identification and crystallinity measurements.
- Similarity indices used in PolySNAP 2.0 and HighScorePlus 3.0 cluster analyses do a good job in separating out cellulose materials based on polymorphism and crystallinity.
PolySNAP – Calculated Fits

- Zero Shift correction
- Autoscaling
- **Automated background subtraction**
- Forces fit to set number of references – but fundamentally unlimited in number, algorithms choose which ones to use
Fibers 81.7%  

Linesheet A 71.33%  

Crystallinity, 50 A Cell 1b  

Ground Pulp 14.5%  

Linesheet C 71.6%
PolySNAP – Pattern summations
Great fit with 98.7 R2, 35 A Cell Ib

Sigma Aldrich 00-060-1502

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percent</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>00-056-1718</td>
<td>82.60</td>
<td>1.10</td>
</tr>
<tr>
<td>00-060-1501</td>
<td>17.40</td>
<td>0.60</td>
</tr>
</tbody>
</table>

CHandsheet

SigmaCell
Nanocrystalline cellulose (30 Å)

Amorphous Cellulose
cellulose II compared to amorphous cellulose

35, 25, 10 Å Cellulose II compared to Amorphous cellulose (top green)
More Conclusions

- Under 30 Å distinctions between the diffraction patterns blur
- Cellulose 1 alpha and 1 beta are highly correlated (dmax 3.82 and 3.87)
- Cellulose II and amorphous cellulose are highly correlated (dmax 4.45 and 4.48)
- Grind cellulose Ia, 1b see the amorphous "jump" but not with cellulose II
Microcrystalline Cellulose Sigma Alrich (00-060-1502) and US Pharmacopeia
Microcrystalline Cellulose

- Predominately Cellulose I beta (XRD)
- ~ 40 A Crystallites (XRD)
- 20 um particles (Sigma–Alrich specification)
- ~ 3 % absorbed water at RT (DTA)
- 1–3 % amorphous cellulose (XRD–FULLPat)
  (other programs estimate 10–20%)
- Microcrystallinity confirmed by PDF analysis showing long coherence lengths and bond distances typical of Cellulose I’s

Suggested by published studies
- The particle size and crystallite size are in the known magnitude of the fibril and microfibril widths, respectively
- Derived from native cellulose the microcrystalline cellulose may have polymorph 1 alpha on fibril surfaces, XRD may not detect a few %
Cellulose
Hickory
Redwood
Lignum Vitae

Yellow Mahogany, Butternut
8 New Reference Materials
2006–2011

In progress (ICDD grant data, collected, being processed for publication)
Cellulose triacetate (USP), microcrystalline cellulose (USP),
Cellulose acetate pthalate, cellulose acetate butyrate – both amorphous
(Support elemental analyses, DSC, DTA)
Povidone, crospovidone

Roman Shpanchenko, Moscow State University

Pair distribution function analysis of all in-progress materials
Valeri Petkov, Central Michigan University
Substituted Celluloses

June Turley, Dow Chemical, 1965

3 Reaction sites
Crystallinity affects
Chemical accessibility
Substitution in Cellulose

- 3 – OH sites per glucose monomer
- Unsubstituted (0) and fully substituted (3)
- 3 mono substitution choices (site 1, 2, 3)
- 3 disubstituted choices (1,2…1,3 …2,3)
New substituted Celluloses

Triacetate = grey
Amorphous cellulose = black
Acetate pthalate = blue
Acetate butyrate = red
Substituted Cellulose (Not always amorphous)

Oriented Film then slowly annealed

Tom Blanton
Eastman Kodak

Crystalline cellulose triacetate compared to USP cellulose acetate butyrate
1. Ab-initio structures used to calculate cellulose polymorphs powder pattern references have been validated in the study of pulps and papers to aid in the determination of polymorphic composition.

2. Amorphous cellulose references have similarly been validated and can be used in the determination of crystallinity.

3. Using the references, a wide variety of cellulose containing material have been studied, polymorphs analyzed, and crystallinities measured.
CONCLUSIONS – Methods

- **Cluster Analyses** – Have been shown to be very valuable in separating out clusters of cellulose containing materials based on polymorphism and crystallite size.

- **Integral Index** – A nice tool for non-crystalline and small crystallite materials to identify phase and polymorphism. Has an advantage when applied to subfiles.

- **Rietveld** – May be too powerful for these relatively simple patterns too many refined variables with too little data. Often refines to an averaged structure with a small crystallite size when other data may indicate a polymorphic mix. Best used with the highest quality data (i.e. synchrotron) and/or with constrained refinement.

- **Pattern Fitting Methods** – Three different programs used, often worked well for crystallinity measurements and polymorphic identification. These methods are very dependent on using the correct crystallite size for the references. This require reiteration – pattern fit, adjust crystallite size, pattern fit again.

- *All methods* were **highly dependent** on accurately removing background and cleanly separating background from amorphous or microcrystalline contributions. This also means that specimen preparation and data collection methods must be reproducible and aimed at reducing background effects as much as possible.
Cellulose is wonderfully versatile and chemically complex – it will provide work for scientists for generations to come

Most wood pulps, pharmaceutical cellulose and paper pulps can be described as a mixture of cellulose 1a, 1b and amorphous cellulose.

The most common combination for commercial materials, made from cotton and wood, is a high cellulose 1b content (>60%) with smaller amounts of cellulose 1a and amorphous cellulose.

We can measure polymorph and crystallinity changes in grinding studies and mercerization processes.

Lignum vitae, an extremely hard wood, also appears to be unusual in that it is predominately cellulose 1a polymorph, several other types of woods also appear to have significant 1a contributions.
ICDD Pattern Summation – Lignum Vitae
Experimental data (red)

Summation (black)
66% Cellulose Ia, 25A
34% Amorphous cellulose

Rietveld, 0.03 pattern shift
A sample of St John’s Wort showing distinct features of Cellulose II (35 Å)
Two Groups of Pulps

35 A Cellulose 1a and 1b

Lignum Vitae
6 hr scan
Samples – Data Collection and analysis

2002–2007

- 12 Pharmaceutical Tablets – Fangling Needham, ICDD clinics, Cam Hubbard, Oak Ridge National Lab, Jim Kaduk, Argonne Light Source
- 18 Wood Pulps, Cotton Linters – Eva Bucher, International Paper

2010–2011

- 21 Wood chips – Jim Kaduk, Poly Crystallography Inc
- 6 USP references – ICDD editors, Joel Reid and Suri Kabekkodu, ICDD grantees, Victor Petkov, Roman Shpanchenko
Challenges

Where is the baseline?

How do you separate Microcrystalline line broadening from the amorphous content or air scatter or Brehmstralung radiation

35 Å cellulose I beta Calculated from Ab-initio structure

Mercerized cellulose
International Paper

Microcrystalline cellulose
Sigma Aldrich

Amorphous Cellulose
International Paper
Polymorph based clusters
Cluster groups from a cluster analysis

1 beta

alpha/beta Blends ??

Amorphous

Mercerized Cellulose II blend
Software Toolkit

Deconvolution Software

**Similarity Indices**
Cluster Analyses
(PANalytical HighScore Plus 3.0.2)
Similarity Index
(ICDD PDF-4 Release 2011)
PolySnap
(Bruker-AXS Version 2.0)

**Refinements**
Rietveld Refinement
LeBail Refinement
Pattern Fitting (FULLPat)
(PANalytical HighScore Plus 3.0.2)

**Pair Distribution Functions**
RAD – Valeri Petkov

**Pattern Summation** – ICDD Release 2011
Confidence in Standards Experimental Data compared to pattern fit from standards

Ground pulp with amorphous/1 beta blend

SigmaCell with amorphous/1a/1b

Mercerized pulp – II/1a/amorphous

Ground pulp – 1b and amorphous
Step 3a. Added in a very small size Cell 1a (width = 1.86), increases

Best fits with a Cellulose I mix of Alpha and beta
55% Cellulose Ib
15% Cellulose 1a
30% Amorphous
Using Kaduk 1beta and 3 hr as reference pts  
Crystallinity calculated as 24.2 % for the 1 hour grind
Pattern Fitting - Mercerized pulp
FULLPat in Highscore Plus

1 hour mercerized, 6% crystallinity left
Fit and residual good

6% Crystallinity
## Best Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>% Cry</th>
<th>Polymorph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigmacell</td>
<td>70 %</td>
<td></td>
<td>54 lb</td>
</tr>
<tr>
<td>Sigmacell</td>
<td>1 hr</td>
<td>24.2%</td>
<td>24.2 lb</td>
</tr>
<tr>
<td>Sigmacell</td>
<td>2 hr</td>
<td>3 %</td>
<td></td>
</tr>
<tr>
<td>Sigmacell</td>
<td>3 hr</td>
<td>0 %</td>
<td></td>
</tr>
<tr>
<td>Mercerized*</td>
<td>70 %</td>
<td></td>
<td>30 lb</td>
</tr>
<tr>
<td>Mercerized</td>
<td>10 min</td>
<td>32 %</td>
<td>8 lb</td>
</tr>
<tr>
<td>Mercerized</td>
<td>1 hr</td>
<td>6 %</td>
<td>1 lb</td>
</tr>
</tbody>
</table>

* Statistically poor fit
Pattern Fitting – Cotton Linters
Rietveld

Cellulose I beta
Butternut: Small crystallite size shifted unit cell a lot

Not much difference Between using a 1b model with Unit cell shift and a mixed Ia/ib model both had Rf below 5
ICDD Pattern Summation 80/30

Raw Data Lignum Vitae
25 Å Cellulose Iα
Amorphous Cellulose
Blue is cellulose Ia and cellulose Ib characteristics.
Green is cellulose II characteristic.
Yellow is cellulose III characteristic.
**Red are substituted cellulosics** – generally peaks at lower angle (triacetyl, tripropionate, nitrate, perchlorate, glycerine and trimethyl).
Cluster Analysis Dendrogram – Wood chips, pulps, and papers

Cell 1b large crystallite

Cell 1a/1b mix

Cell 1b Small Crystallite

Cell 1a/1b mix

Cell II/I mix

Amorphous
PolySNAP – 4 Reference Set Used Cell II (red), cell 1b (yellow), cell 1a (green) and amorphous (blue)

Note
Cellulose II associates with amorphous

Highest 1b specimen did not cluster

Cellulose 1a and 1b associate
Hickory, Mahogany and Lignum Vitae

All show intensity around 10 degrees two theta

Hickory and Mahogany both exhibit a peak at 34–35 degrees that is usually associated with cellulose Ib, the pattern looks to be predominately small crystallite size cellulose Ib, but it may be a 1b/1a mix

Lignum Vitae

Exhibits the character of a small crystallite size 1a with an amorphous component

In both specimens it is very difficult to say whether the intensity at 10–12 degrees is from cell Ia or an amorphous contribution. In mahogany and lignum Vitae there does appear to be slight but distinct slope changes
Small crystallites – different polymorphs

Hickory – 58 % Crystalline, 20 A Cell Ib

Maple – 85 % Crystalline 20 A Cell I a
3 phase Fits

- RED CEDAR
- MAHOGANY
- PINE
- MAPLE
- REDWOOD
Highly crystalline cellulose Ib's

Standards and filter paper

Wood pulps

Maple, Cherry, Mulberry

Lignin

Rosewood

Not clustered but mostly Ib

Not clustered but mostly Cellulose II

Cellulose II

Very High Crystallinity Ib’s

Highly crystalline cellulose Ib’s Standards and filter paper

Amorphous