Cellulose Crystallites

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Abstract: This article discusses advances in understanding the structural and physicochemical characteristics of suspensions of cellulose crystallites prepared by acid hydrolysis of natural cellulose fibres. Consideration of recent developments in visualization of crystallite ultrastructure may provide clues to suspension behavior. In addition, novel applications in a diverse range of fields are presented, from iridescent pigments to biomolecular NMR studies.

Keywords: carbohydrates · cellulose · liquid crystals · NMR spectroscopy

Introduction

Almost fifty years ago, the production of colloidal suspensions of cellulose by the sulfuric acid hydrolysis of cellulose fibres was first reported by Ranby.[1] Electron microscope images of the dried suspensions showed rod-like species, often aggregated; electron diffraction from the rods demonstrated that they had the same crystal structure as the original fibres. Development of the acid degradation of cellulose by Battista[2] led to the commercialization of microcrystalline cellulose (MCC). Because of its useful characteristics, including zero toxicity, good hygroscopicity, chemical inactivity, and reversible adsorbency,[3] MCC, derived from high-quality wood pulp, has found widespread use in the multi-billion dollar pharmaceutical industry as a tablet excipient. With its excellent compression, permeability, and binding properties, MCC is utilized extensively as a binder and filler in solid dosage forms.[4, 5] MCC is also widely used in food production, being found in reduced-fat salad dressings, dairy products, frozen desserts, and bakery products owing to its properties as a stabilizer, texturizing agent, and fat replacer.[6] These applications have been described in extensive detail by other authors.[7–10]

Most grades of MCC are made by hydrolysis with hydrochloric acid, and in high shear fields form colloidal dispersions with a range of particle sizes. In contrast, hydrolysis with sulfuric acid can result in the introduction of sulfate esters at the surface of the cellulose crystallites, leading to added electrostatic stabilization of the suspensions. At sufficiently high concentrations of such suspensions, birefringent, ordered, fluid phases were observed.[11] Subsequent work on carefully hydrolyzed and purified samples demonstrated that a chiral nematic-ordered phase formed above a critical concentration.[12]

The spontaneous formation of chiral nematic phases from aqueous colloidal suspensions of cellulose crystallites is reviewed briefly here. Applications to date range from the formation of iridescent films from dried suspensions[13] to the magnetic alignment of proteins in biomolecular NMR spectroscopy.[14]

Native Cellulose as Raw Material for Crystallites

The biosynthesis of cellulose in species, such as cotton, proceeds by the building up of glucose units into long, slender monocrystalline microfibrils. The cellulose molecules in the microfibrils are arranged in parallel with a twofold screw symmetry along their length achieved by β-[1,4]-linkage of D-Glc subunits, and are deposited in a parallel fashion to form larger fibrillar structures. The degree of polymerization (DP) along the chains in native cellulose is very high, with values up to 13000 for native cotton cellulose claimed by some authors.[15] Microfibrils are arranged into lattices within the cell wall; this results in a highly crystalline structure that is insoluble in water and resistant to reagents. However, areas of the lattice contain unstructured regions that are caused by the presence of amorphous cellulose, or which arise as a result of small crystalline units being imperfectly packed together. Non-cellulosic polysaccharides (e.g., hemicelluloses such as xyloglycans, glucomannans, glucuronoxylans) exhibit a strong interaction with the microfibril surface and further lead to an apparent disorder.[16]

Stresses present during biosynthesis have been implicated in the formation of two different "allomorphs" of cellulose: Iα

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and Iβ (which were originally discovered by analysis of spectral line splitting in solid-state ^{13}C cross-polarization/magic angle spinning (CP/MAS) NMR spectroscopy[17] and later confirmed by electron diffraction and FTIR[18]). Both allomorphs possess symmetry that is very close to twofold screw symmetry; this leads to the conclusion that the repeat unit along the chain is cellobiose (i.e., two glucose units). The structure of Iα is defined by a one-chain triclinic and Iβ by a two-chain monoclinic unit cell. Essentially, these structures are very similar and interconversion between them is achieved by the slipping of intersheet hydrogen bonds between cellulose sheets,[19] to give a slightly different pattern of cellobiose repeats. The differences in proportional compositions of Iα and Iβ help explain the diverse structural characteristics seen in different organisms; bacterial and algal celluloses tend to be richer in Iα (e.g., in *Valonia*, the proportion of Iα:Iβ is $\approx$65:35), and higher plants contain a greater relative amount of Iβ.

Results from studies performed on *Microdictyon*[20] demonstrate that it is possible for Iα and Iβ to exist within the same microfibril; however, detailed AFM images have suggested that only the triclinic (Iα) structure is present at the surface of *Valonia* cellulose crystals. Detection of differences in topographical features, that is, the alternating C2–C3 and O5–C5 faces, along the cellulose chain allowed observation of protruding hydroxymethyl groups on the O5–C5 face that could be differentiated from hydroxyls on the C2–C3 face. The particular cellobiose repeat pattern was identified as the triclinic unit cell. Monoclinic Iβ was not observed in these studies, raising the possibility that this allomorph may only be present inside the crystallite structure. Enzymatic degradation studies support this observation,[21, 22] as does electron microdiffraction.[23] Solid-state NMR spectroscopy on sugar-beet pulp has estimated that the average number of cellulose chains in the core of a microcrystal is 25.

Preparation: Individual crystallites are prepared through acid hydrolysis of cellulose-containing materials under strictly controlled conditions of time and temperature. Typically, concentrated mineral acids, such as sulfuric or hydrochloric, are used; the cellulose starting material comes from many sources, including wood pulp,[12] cotton,[24] *Valonia* (green alga),[25] and sugar-beet pulp.[16] The purity of cellulose contained in these materials differs to a great extent, for example, cotton-seed fluff is composed of $\leq$94% cellulose (wood contains $\leq$55%). Initial acid action removes polysaccharide material closely bonded to the microfibril surface, resulting in an overall decrease of amorphous material. Subsequent hydrolysis breaks down those portions of the long glucose chains in accessible, non-crystalline regions. A levelling-off degree of polymerization is achieved; this corresponds to the residual highly crystalline regions of the original cellulose fibre. When this level is reached, hydrolysis is terminated by rapid dilution of the acid. A combination of centrifugation and extensive dialysis is employed to completely remove the acid, and a brief sonication completes the process to disperse the individual particles of cellulose and yield an aqueous suspension (see Figure 1).[12] The cellulose rods that remain after this treatment are almost entirely crystalline and as such are termed "crystallites".

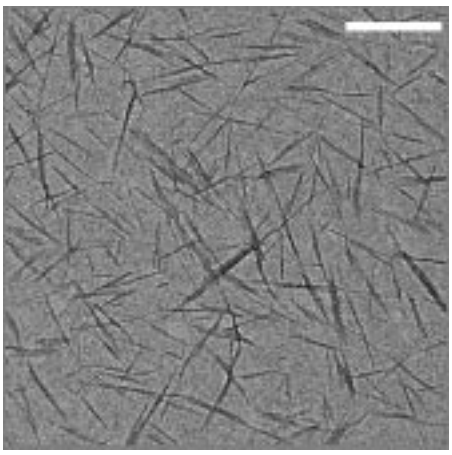

Figure 1. Positively stained electron micrograph of cotton cellulose crystallites (derived from Whatman filter paper) at a concentration of $\approx$0.1% (w/w). The scale bar corresponds to 400 nm.

The precise physical dimensions of the crystallites depend on several factors, including the source of the cellulose, the exact hydrolysis conditions, and ionic strength. Additionally, complications in size heterogeneity are inevitable owing to the diffusion-controlled nature of the acid hydrolysis. Typical figures for crystallites derived from different species vary: $20 \times (100-2000)$ nm for *Valonia*,[26] $(3-5) \times 180 \pm 75$ nm for bleached softwood kraft pulp,[27] and $7 \times (100-300)$ nm for cotton.[28] The high axial (length to width) ratio of the rods is important for the determination of anisotropic phase formation.

Ultrastructure: Beyond the nanometre resolution afforded by electron microscopy, the ultrastructure of individual crystallites has only recently been investigated thoroughly. Indeed, several techniques have provided information at the subnanometre level, including high resolution solid-state NMR spectroscopy,[16] atomic force microscopy (AFM),[29, 30] small angle neutron scattering (SANS),[31] and small angle X-ray scattering.[32, 33] AFM has perhaps provided the most detailed description so far, with a high-resolution picture of the surface of cellulose crystallites derived from a green alga, *Valonia* (see Figure 2). Topographical images gained by using AFM have revealed corrugations across the surface of each crystal, with three spacings relating to the 0.52 nm glucose interval, the 1.04 nm cellobiose repeat distance, and an $\sim$0.6 nm repeat matching the intermolecular spacing between chains (see Figure 3). This represents the first direct imaging of crystallographic features on the surface of cellulose crystallites.

At present, the internal structural features of cellulose microcrystals remain undetermined, although some NMR spectroscopic evidence indicates that the interior may exhibit a greater degree of organization.[34, 35] The studies performed to date, however, have allowed a greater understanding of cellulose crystallite ultrastructure, with a view to understanding the fundamental characteristics exhibited by such suspensions.

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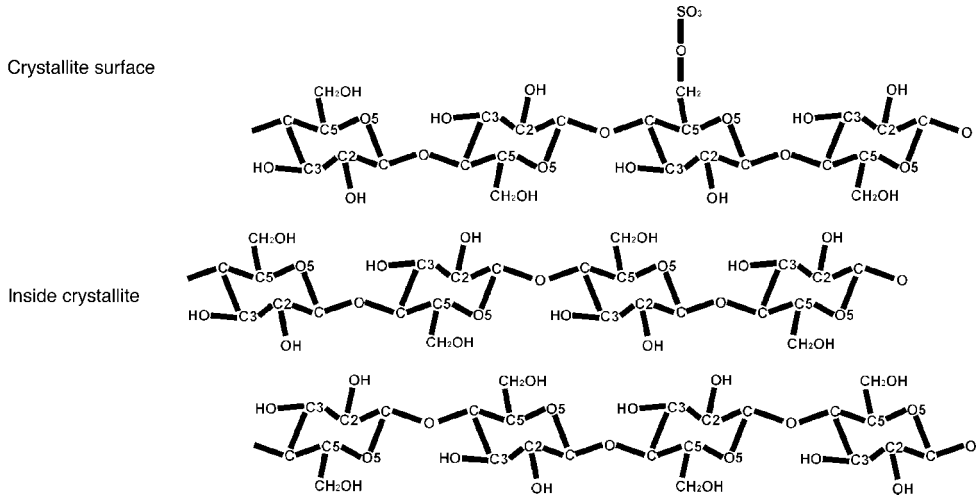

Figure 2. Backbone trace of several cellulose chains in the monoclinic (Iβ) conformation, showing the alternation of O5–C5 and C2–C3 faces at one surface. Approximately one in ten surface hydroxymethyl groups are substituted with sulfate esters.

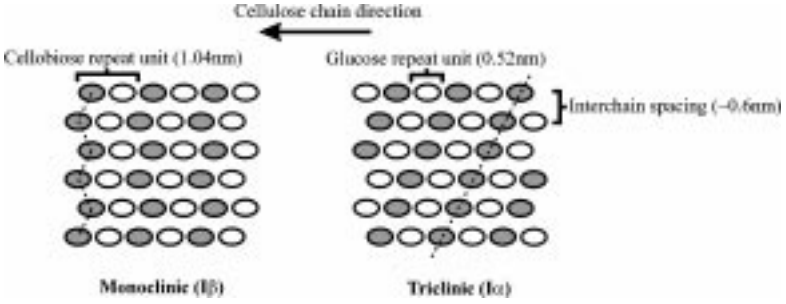

Figure 3. Schematic diagram of the two allomorphs of cellulose I present in crystallites. Each oval represents a glucose sugar unit. The difference in alignment of adjacent chains leads to either a staggered pattern in the monoclinic (Iβ) unit cell or a diagonal pattern in the triclinic (Iα) unit cell.

Chiral nematic properties: Rod-shaped species, for example, tobacco mosaic virus,[36] fd phage,[37] and poly(tetrafluoroethylene) "whiskers"[38] have been demonstrated to display *nematic* order, whereas suspensions of cellulose crystallites spontaneously form a *chiral nematic* phase.[12] The chiral nematic, or cholesteric, phase consists of stacked planes of molecules aligned along a director (***n***), with the orientation of each director rotated about the perpendicular axis from one plane to the next, as shown in Figure 4a. The source of the chiral interaction is thought to arise from the packing of screwlike rods (see Figure 4b), as postulated by Straley's hypothesis,[39] and indeed a SANS study is claimed to support this.[31]

Interestingly, the phase-forming ability of a cellulose crystallite suspension depends on the mineral acid chosen for the initial hydrolysis. Use of either sulfuric or phosphoric acid yields a chiral nematic phase, but hydrochloric acid hydrolysis gives a viscous suspension that forms a birefringent, glassy phase after a "post-sulfation" treatment.[40]

For crystallites prepared from the action of concentrated sulfuric acid on bleached kraft wood pulp, the axial ratio of the rods is in the range of 20 to 40. According to phase equilibrium theory for rodlike particles,[41] this should correspond to a critical concentration for ordered phase formation of 0.2 to 0.5. However, phase separation has been observed at an approximately tenfold lower, 1–2%, volume fraction. Elemental analysis performed on these crystallites has offered an explanation for this phenomenon, through anionic stabilization due to attraction/repulsion forces of electrical double layers.[11] The analysis revealed a sulfur content of 0.73% (w/w), which suggests sulfur deposition on the surface of the rods as a result of a side reaction during hydrolysis. In fact, the acid reacts with hydroxymethyl groups at the crystallite surface to form sulfate esters, and it has been estimated that $\approx$10% of the anhydroglucose units on the surface contain sulfur. Post-sulfated hydrochloric acid preparations have a sulfur content approximately one third less than this,[40] and thus provide further evidence that charge on the crystallite surface is imperative for phase stability. Quantitative interpretations of the changes in composition of the isotropic and anisotropic phases in the concentration range in which both phases coexist have been attempted as a function of electrolyte concentration[42] and nature of the counterion.[43] Interestingly, the chiral nematic pitch was found to decrease (the phase became more highly twisted) as the salt concentration increased.[42] Recently, Heux et al.[44] succeeded in preparing cellulose crystallite suspensions in organic media, stabilized by surfactant, and found evidence for chiral nematic ordering.

The self-assembly of chiral nematic phases by suspensions of cellulose crystallites has received an increased amount of attention for its potential applications. Chiral nematic liquid crystals whose pitch is of the order of the wavelength of visible light reflect circularly polarized light of the same handedness as the chiral nematic phase.[45] The wavelength of this selectively reflected light changes with viewing angle, leading to an iridescent appearance. That cellulose derivatives can form iridescent liquid and solid phases has long been known.[46] By simply casting films from suspensions of cellulose crystallites, cellulose films with the optical properties of chiral nematic liquid crystals can also be prepared.[13]

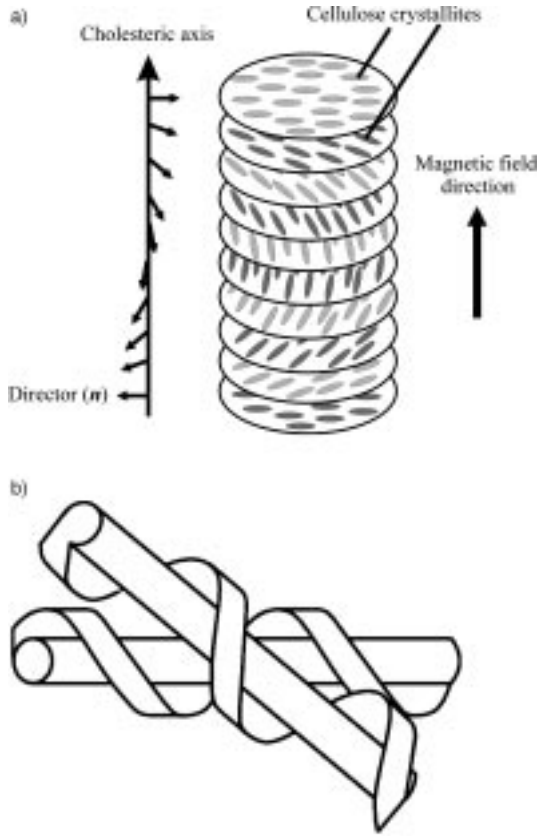

Figure 4. a) Schematic representation of the chiral nematic phase. Each nematic plane, containing aligned, rodlike cellulose crystallite molecules, exhibits an orientation that differs slightly from adjacent planes due to rotation of the magnetic director (***n***) about the perpendicular cholesteric axis. b) Schematic representation of the tight packing achieved by chiral interaction of screwlike rods. Alignment of the "thread" of one rod with the "groove" of its neighbour results in closer packing than is observed if the long axes of the rods are parallel to one another.

Tailoring of the films to give different colors of reflected light was achieved by altering the salt content of the suspension for a given source of cellulose and set of hydrolysis conditions. Possible areas of application include optically variable films and ink pigments for security papers, as the optical properties cannot be reproduced by printing or photocopying.[47]

While cellulose crystallite suspensions of low ionic strength allowed to dry in a relaxed state usually take a chiral nematic structure, evaporation *under shear* of a suspension of cellulose crystallites from the green alga *Cladophora* sp. gave a film with highly oriented uniaxial structure.[48]

Alignment in a magnetic field: When a suspension of tunicate cellulose microcrystals from *Halocynthia roretzi* was allowed to dry in a homogeneous 7 T magnetic field, the crystallites exhibited an alignment with their long axes perpendicular to the direction of the field, indicating a negative diamagnetic susceptibility anisotropy.[49] Since the particles re-oriented across, rather than along the field, the field does not untwist the chiral nematic structure, but rather lines up the chiral nematic axis along the direction of the field.[24, 50] The process of magnetic alignment occurs over a period of hours to days owing to the viscosity of cellulose crystallite suspensions, and, therefore, applications in areas such as liquid crystal displays are unlikely. However, immediate alignment is not always a requirement. For example, reinforced polymers may be achieved through polymerization within a magnetic field, and increased strength is a characteristic which is much sought after in the textile industry.

Recently, a novel application in the field of structural biology makes use of both the anisotropy of the cellulose crystallite, the stability of the suspensions, and the orientation of the suspension in a magnetic field. Elucidating the structure of nucleic acids and proteins is one of the key activities in molecular biology. Nuclear magnetic resonance (NMR) spectroscopy is a technique that may be used for the elucidation of three dimensional macromolecular structure in aqueous solution. One of the limiting factors for this technique is the size of the macromolecule studied. However, the application of several liquid crystal phases, including fd phage[37] and surfactant bicelles,[51] has led to improvements in both structural precision and the maximum size of proteins amenable to study. The small degree of molecular alignment that these dilute phases induce in solution allows the measurement of residual dipolar couplings, a phenomenon not normally observed under isotropic conditions. Use of aligning phases is likely to become standard in NMR structural determination. There is a requirement for a ubiquitous medium in which to align the macromolecules, yet media described to date have various disadvantages. For example, phage show a degree of interaction with charged areas of proteins, and bicelles, which are stable over narrow temperature ranges, exhibit a delicate phase equilibrium that may be disrupted by the presence of soluble macromolecules. However, cellulose crystallites have been demonstrated to fulfil the role of molecular alignment,[14] yet show no interaction with a highly basic protein (intimin, $pI = 8.9$), despite their negative surface charge (see Figure 5).

In addition, the observation that cellulose crystallites align perpendicular to the direction of a magnetic field is in direct

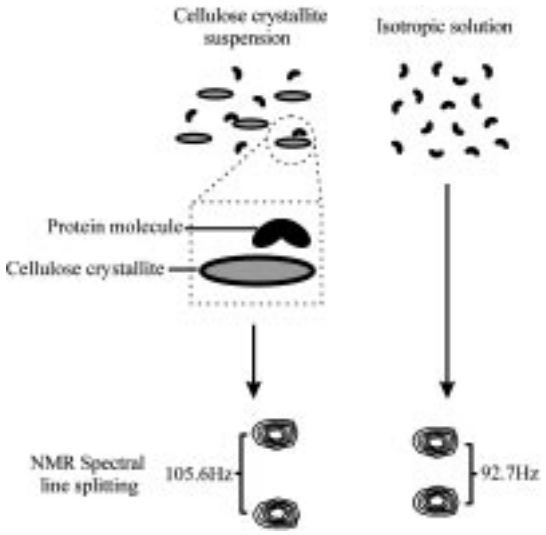

Figure 5. Schematic showing alignment of macromolecules in a magnetically aligned anisotropic phase of cellulose crystallites. Molecules in the vicinity of cellulose crystallites experience preferential alignment (represented in the broken box). This results in a change in spectral line splitting, for example from 92.7 Hz to 105.6 Hz.

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contrast to existing alignment technologies, all of which exhibit a positive diamagnetic susceptibility anisotropy and, therefore, align parallel to the field.

Conclusion

Cellulose crystallites in the form of microcrystalline cellulose are currently utilized widely industrially. However, as yet unexploited features, such as the ability to form chiral nematic phases at relatively low concentrations in water and the alignment of such suspensions in magnetic fields, has paved the way for future applications in other diverse areas. Central to this expansion will be an improved appreciation of the structure of the crystallites themselves and the factors that govern the stability and structure of the ordered phases. The desirable characteristics of cellulose crystallites, such as the abundant and renewable nature of the starting material, their inertness and absence of toxicity, and the relative stability of the suspensions suggest that this media is likely to find an even wider range of uses in the near future.

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