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# Characterisation of large scale structures in starch granules via small-angle neutron and X-ray scattering

James Doutch, Elliot P. Gilbert*

*Bragg Institute, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, NSW 2232, Australia*

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ABSTRACT

Small angle scattering (SAS) techniques have a distinguished track record in illuminating the semi-crystalline lamellar structure of the starch granule. To date, there have been few attempts to use SAS techniques to characterise larger-scale structures reported from imaging techniques such as growth rings, blocklets or pores, nor how these structures would modulate the well-known scattering arising from the semi-crystalline lamellar structure. In this study, SAS data collected over an extended $q$ range were gathered from dry and hydrated starch powders from varied botanical sources. The use of neutrons and X-rays, as well as comparing dry and hydrated granules, allowed different levels of contrast in scattering length density to be probed and therefore selected structural regions to be highlighted. The lowest $q$ range, 0.002–0.04 Å$^{-1}$, was found to be dominated by scattering from the starch granules themselves, especially in the dry powders; however an inflection point from a low contrast structure was observed at 0.035 Å$^{-1}$. The associated scattering was interpreted within a unified scattering framework with the inflexion point correlating with a structure with radius of gyration ~90 Å – a size comparable to small blocklets or superhelices. In hydrated starches, it is observed that there is an inflection point between lamellar and $q^{-4}$ power-law scattering regions at approximately 0.004 Å$^{-1}$ which may correlate with growth rings and large blocklets. The implications of these findings on existing models of starch lamellar scattering are discussed.



## 1. Introduction

Starch is the primary carbohydrate component in the human diet and the major storage polysaccharide in plants. Structurally it is deposited in a large number of granules which vary in dimension (~1–100 μm) according to botanical source (Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994). These granules are partially crystalline in nature containing alternating amorphous and semi-crystalline layers termed growth rings. The semi-crystalline layers consist of a lamellar arrangement of crystalline and amorphous regions with repeat distance of 90–100 Å that is readily detected in hydrated starch by small angle scattering (SAS) techniques, using X-rays and neutrons (SAXS and SANS) (Blazek & Gilbert, 2011; Cameron & Donald, 1992; Waigh, Gidley, Komanshek, & Donald, 2000). It is proposed that this occurs as the amorphous regions within the lamellar structure preferentially take up water and, as a result, an observable contrast in scattering length density between the crystalline and amorphous regions is achieved. However, in dry powders, the scattering associated with this lamellar peak is significantly decreased, as the intensity is largely based on intrinsic differences in physical density between the crystalline and amorphous regions (Cameron & Donald, 1992; Waigh et al., 2000). This is shown in Table 1 where the square of the contrast in scattering length density, for both neutrons and X-rays, for starch granules and their internal regions with respect to air and $H_2O$ are summarised. X-ray powder diffraction techniques, characterising starch at spacing smaller than 1 nm demonstrate the presence of a number of different crystalline polymorphs at these length scales, such as the classic A- and B-type patterns. This shows considerable variance between granule species; for example, many cereal starches show A-type diffraction patterns, whereas the tuber and high amylose starch types often display B-type patterns (Buleon, Colonna, Planchot, & Ball, 1998).

The scattering from dry starch powders has not been studied in depth (Cameron & Donald, 1992; Waigh et al., 2000) and has been rationalised using two complementary approaches. The first assumes that there are three distinct scattering length densities within the granule (Cameron & Donald, 1992): corresponding to (i) crystalline and (ii) amorphous regions within the semi-crystalline lamellae and, surrounding these semi-crystalline lamellae, are (iii) amorphous growth rings. It is therefore assumed

* Corresponding author. Tel.: +61 2 9717 9470; fax: +61 2 9717 3606.
*E-mail address:* elliot.gilbert@ansto.gov.au (E.P. Gilbert).

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**Table 1**
The square of the scattering contrast for SAXS and SANS of starch granules, assuming granule density 1.50 g cm$^{-3}$, amorphous fraction density 1.59 cm$^{-3}$ and crystalline fraction density 1.72 g cm$^{-3}$ (1).

| | X-ray ($\times 10^{20}$ cm$^{-4}$) | Neutron ($\times 10^{20}$ cm$^{-4}$) |
|---|---|---|
| Overall granule to air | 182.3 | 3.08 |
| Overall granule to water | 16.66 | 5.36 |
| Crystalline fraction (hydrated) to water | 36.74 | 6.62 |
| Amorphous fraction (hydrated) to water | 23.93 | 5.86 |

that if the starch granules are not hydrated in excess water, there is insufficient contrast between the amorphous and crystalline regions for the lamellar peak to be observed with respect to the surrounding amorphous growth ring. The second approach considers starch to act as a liquid crystal, with hydration enabling sufficient chain mobility to occur such that a proportion of the amylopectin branches are able to align (Waigh et al., 2000). Conversely, in the absence of water, the branches exhibit greater disorder and lamellar scattering is reduced. This is often considered within the formalism of nematic and smectic behaviour – in the smectic state helices are aligned into lamellae, with this breaking down into a laterally non-aligned, smectic, state.

These structural models provide satisfactory fits for the lamellar peak, however do not account for scattering observed at q values below the lamellar peak, i.e. <~0.04 Å$^{-1}$ (Blazek & Gilbert, 2010, 2011). This may imply that the SAS patterns are modulated at these $q$-ranges by other larger scale structures not accounted for in a purely lamellar description. For example, Cameron and Donald (1992) interpret the scattering from potato starch to be associated with semi-crystalline growth rings that, on average, contain ca. 16 lamellar layers of approximately 90 Å each, giving a total thickness of ca. 1440 Å. However, such large-scale structures would be expected to influence the scattering pattern down to ca. 0.004 Å$^{-1}$. Surface pores are thought to have diameter of 20–30 Å (Sujka & Jamroz, 2010); scattering from these structures is likely to be masked by lamellar scattering. However, a population of larger cavities or macroscopic pores of dimension 1000–2000 Å may occur around certain granules, which could affect scattering in the range ca. 0.006–0.003 Å$^{-1}$. The influence of pores may become increasingly significant in the scattering from starch granules subject to enzymatic hydrolysis.

The use of imaging techniques, such as atomic force microscopy, on starch granules has suggested the presence of an intermediate level of order within the starch granule that is not accounted for within existing scattering frameworks. These are referred to as blocklets and are believed to form spheroidic structures within the growth rings. Reported dimensions of these structures are in the range 150–5000 Å and which appear to vary in location within a granule and with botanical source (for review see Perez & Bertoft, 2010; Tang, Mitsunaga, & Kawamura, 2005). Other substructures which may plausibly modulate the scattering pattern are superhelices (Oostergetel & van Bruggen, 1993).

In this study we utilised SAXS and SANS to investigate the scattering from starch in the form of 'dry' powders (i.e. those at their ambient, native hydration ~15%) and from fully hydrated starch slurries (using $H_2O$ for both X-ray and neutron studies) to investigate the low $q$ scattering under different contrast conditions in the context of considering the influence of structures in the starch granule on larger length scales than lamellae. One key advantage of the use of SANS and SAXS techniques is the minimal sample preparation required, and in particular the ability to avoid fixatives and penetrating resins.

## 2. Materials and method

### 2.1. Small angle X-ray scattering (SAXS)

SAXS measurements were performed on a Bruker Nanostar with rotating anode source operated at wavelength, λ of 1.54 Å (Cu $K_\alpha$) with a sample-to-detector distance of 115.5 mm, and beam size 0.5 mm, giving $q$ range from 0.005 to 0.22 Å$^{-1}$ using a Vantec 2000 2D area detector, where $q$ is the magnitude of the scattering vector defined as:

$$q = \frac{4\pi}{\lambda} \sin\,\theta \tag{1}$$

and where $2\theta$ is the scattering angle.

Native starch powders from tapioca (Penford, AU), maize (Penford, NZ), waxy maize (Tate and Lyle, Decatur, IL), high amylose maize (Hylon VII, Penford, AU), potato, and wheat (both commercial material, supermarket, Sydney, AU) were presented in 2 mm quartz capillaries (Hilgenburg Gmbh, Germany) as both 'dry' powders at ambient hydration levels and as hydrated slurries under excess water to the X-ray beam. The scattering patterns were reduced and radially averaged with Bruker software. A scattering background from an empty quartz capillary, or quartz capillary filled with water, were subtracted as appropriate, after correction for sample transmission. X-ray data were placed on an absolute scale using water as a standard (Fan, Degen, Bendle, Grupido, & Ilvasky, 2010). These operations were performed using the IRENA macro suite for manipulating and analysing SAS data (Ilavsky & Jemian, 2009) within the Igor analysis package (Wavemetrics, Lake Oswego, OR). We note, however, that absolute intensity values are highly influenced by the packing density of the samples which is ill-defined.

### 2.2. Small angle neutron scattering (SANS)

SANS experiments were performed on the 40 m Quokka instrument at the OPAL reactor; this instrument has been described previously (Gilbert, Schulz, & Noakes, 2006). Four configurations were used to cover a $q$ range 0.0005–0.7 Å$^{-1}$. Three of these were 'standard' configurations: (i) source-to-sample distance (SSD) = 9.965 m, sample-to-detector distance (SDD) = 1.354 m, (ii) SSD = 3.949 m, SDD 4.044 m and (iii) SSD = 20.245 m, SDD = 20.094 m using a wavelength of 5.034 Å and 14% wavelength resolution and with source and sample aperture diameters of 50 mm and 10 mm, respectively. To access lower $q$ values, a focussing optics configuration was used in which an array of 24 $MgF_2$ lenses were employed to focus neutrons at the detector with wavelength 8.1 Å; in this approach, minimum $q$ is determined by the size of the image of the source aperture at the detector. As with the SAXS measurements, both dry and hydrated samples were investigated, having first been placed in 1 mm path length cells with demountable quartz windows.

SANS data were reduced using NCNR SANS reduction macros (Kline, 2006) modified for the Quokka instrument in Igor. Background subtraction and sample transmission corrections were performed using an empty cell or a cell of water as appropriate. Data were then transformed to absolute scale by the use of an attenuated direct beam transmission measurement.

### 2.3. Data analysis

SAXS and SANS data were plotted in the IRENA package (Ilavsky & Jemian, 2009). Selected data are displayed in the form of $I(q)q^4$ plots to emphasise deviations from Porod-type scattering (i.e. $I(q) \sim q^{-4}$) arising from granule–solvent or granule–air interfaces. Data were analysed using the Beaucage Unified fit method (Beaucage, 1996) as outlined in Section 4 and coded within the NIST analysis macro suite (Kline, 2006).