Giant Amyloid Spherulites reveal their true colours

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1. Introduction

Amyloid spherulites are protein aggregates that have been widely studied because of their association with pathological conditions such as Alzheimer's and Parkinson's disease.

Spherulites are typically 5-30μm in size and self-assemble from individual proteins under denaturing conditions to form radially oriented fibres.

Here we report on "Giant Amyloid Spherulites" (GAS) which may grow up to ~1 mm in diameter and produce coloured rings when observed under crossed polarisers.

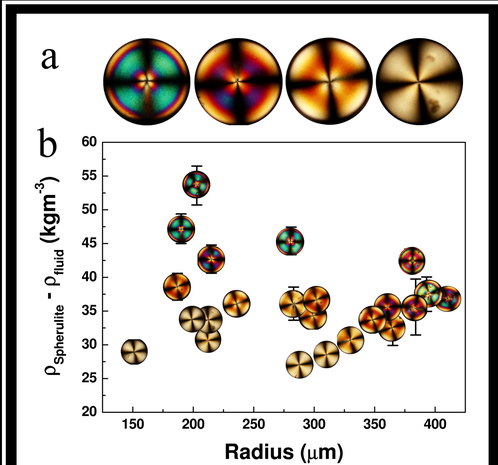

Fig 1 - a) Giant Spherulites viewed between crossed polarisers b) Patterns observed for spherulites of given density and radius

2. Experiment

GAS were formed from Bovine Insulin (10mgml^{-1}), 25mM NaCl, pH 2.8 HCl, by Incubating at T=67°C for 24 hrs.

GAS were viewed between crossed polarisers with an optical microscope in transmission mode.

Some spherulites displayed beautiful coloured rings, whilst others of comparable size, from the same samples, were uniformly white (Fig 1a)

The density of individual spherulites obtained from measurements of sedimentation velocity was correlated with changes in the coloured rings ("Isochromes")

Increasing radius also resulted in differences in the patterns observed (Fig1b).

"Giant Amyloid Spherulites" (GAS) were placed in Water-Glycerol mixtures of different refractive index.

As the index mis-match was decreased (Higher n_{liq}) the GAS became less colourful (Fig 3)

Investigation with our model suggests that these changes result from differences in the form birefringence (see Fig 2).

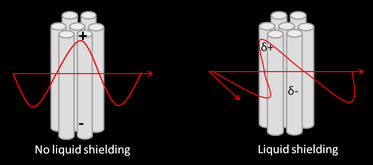

Fig 2. Schematic illustrating the origins of form birefringence

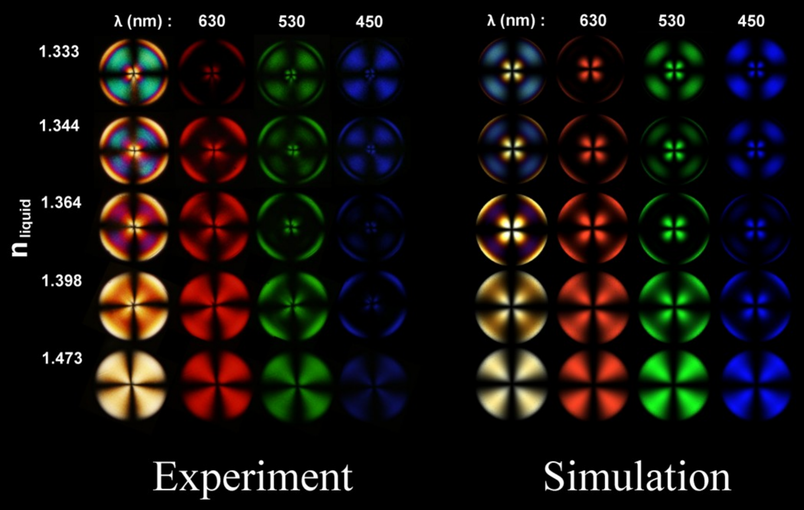

Fig 3. The same spherulite suspended in water-glycerol mixtures of different refractive index, and viewed with narrow bandpass filters at the wavelengths shown.

3. The Model

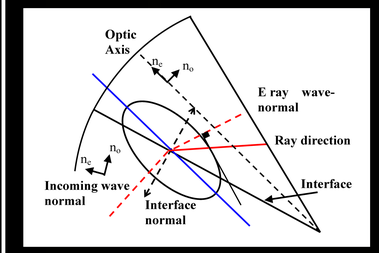

Fig 4. Rays undergo refraction at each interface.

Rays exiting the disc are refracted at the liquid air interface.

Virtual rays are then projected back to the object plane (S in Fig 5) to form an image.

Rotating this disc about the z axis (see Figure 6), a complete image of the spherulite is formed.

We model 2000 rays through a disc composed of 1000 segments. Each segment has an ordinary (n_o) and an extraordinary (n_e) refractive index (See Fig 4 & 5).

The refractive index and propagation direction of each ray are calculated at each segment interface. The path length for each ray is also calculated.

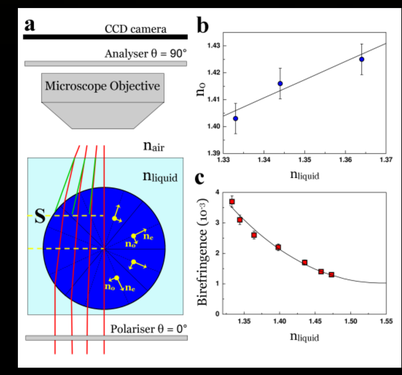

Fig 5 a) Schematic of model. b & c) Estimates of refractive index and birefringence derived from images in fig 2.

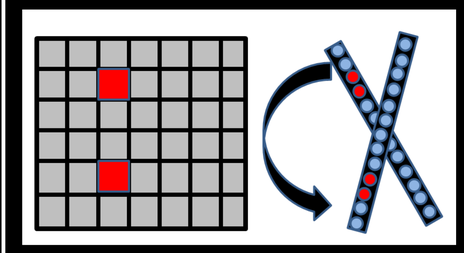

Fig 6. Rays from the disc are mapped to pixels to produce a full spherulite image.

Using the experimental images in fig 3 we estimate the refractive index and birefringence of a GAS using our model.

Fitting this data we can estimate the volume fraction of protein and contributions of form and intrinsic birefringence.

The results of our simulation are shown in fig 3. We see good agreement, suggesting that our model captures the essential physics of how polarised light interacts with the GAS structures to produce the isochromes.

4. Conclusions

The colourful spherulites patterns observed under cross polarisers arise from a combination of birefringence, refraction and the spherical geometry.

Using a combination of experiment and a ray tracing model we have explained the origin of these patterns.

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