ESRF	**Experiment title:** Liquid-crystalline collagen: Structure and thermodynamics of the cholesteric phase	**Experiment number**: SC1642
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Report:

Collagen is one of the most abundant and ubiquitous protein of the animal kingdom, representing about 1/3 of all human proteins. In connective tissues, and in particular skeletal materials, it plays a considerable role as a structuring organic component to which a mineral phase of calcium phosphate is often closely associated. Type I collagen is for instance the principal protein forming the organic fibrous ordered network in bones, skin, ligaments, tendon and cornea.

Our main objective during the run on ID02 in July 2005 was to assess the nematic nature of anisotropic concentrated collagen solutions. Studies of the phase transition in polarized light indicated that the transition threshold is situated near 100 mg/mL. In some cases, coexistence between the two phases, isotropic and nematic, could be observed, which suggested that the transition is first-order and that concentrated collagen mostly behaves like a dispersion of rods, as theoretically described by Onsager. Because of the relative weakness of the signal, the high flux of the beamline and the sample environment available on ID02 (cylindrical geometry shear cell, SAXS/WAXS) was necessary to analyze the structure of the nematic phase with strongly shear-aligned samples. In this configuration, we were able to reach a good enough signal-to-noise ratio to monitor an interference peak arising from interactions between neighbouring collagen molecules in both disordered and ordered liquid phases.

Nematic phases (w%>ca 8) under shearing in a Couette cell exhibit a rather broad correlation peak at low q-values. Shearing is used to align the sample and produce nematic monodomains. Collagen molecules are oriented in the direction of shear (horizontal), which produces a strongly anisotropic signal along the vertical direction (Fig. 1a). Isotropic phases also give rise to small-angle scattering either at rest (Fig. 1b) or under shearing (Fig. 1c) and display a correlation peak (Fig. 2) at a q-vector smaller than for the nematic phase. An isotropic sample at rest shows no alignment, the pattern is centrosymmetrical and the signal is too weak to locate an intensity maximum. Under shearing, the signal gathers along the vertical direction and a correlation peak is visible. Contrary to nematic solutions, an isotropic sample relaxes back to a unaligned state as soon as the shearing is stopped (Fig. 1b).

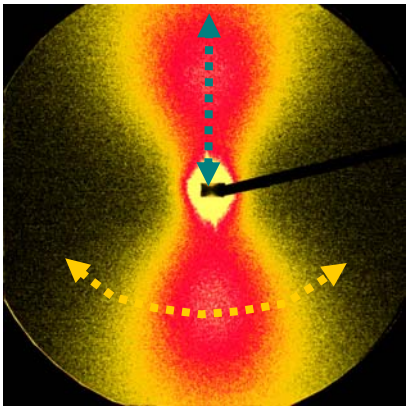

Fig 1a : nematic sample under shearing ($\gamma\bullet$ = 8 s^{-1}, D=3 m, w%=9)

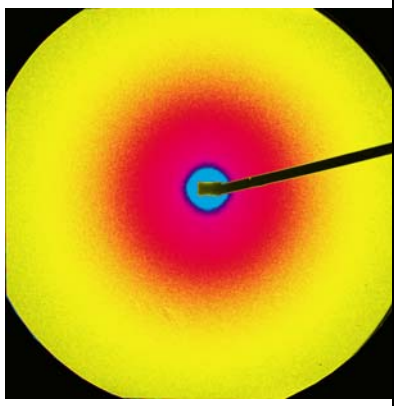

Fig 1b : relaxed isotropic sample ($\gamma\bullet$ = 0 s^{-1} D=3 m, w%=4)

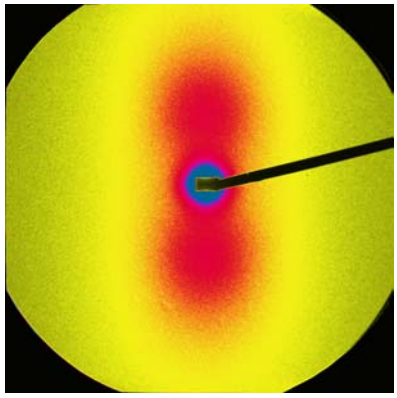

Fig 1c : same isotropic sample under shearing ($\gamma\bullet$ = 400 s^{-1} D=3 m, w%=4)

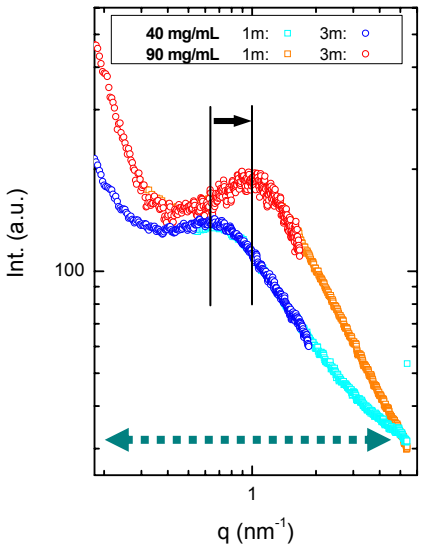

Fig 2 : Linear profiles along the vertical direction of SAXS patterns showing that q_{max} increases when the sample concentration increases.

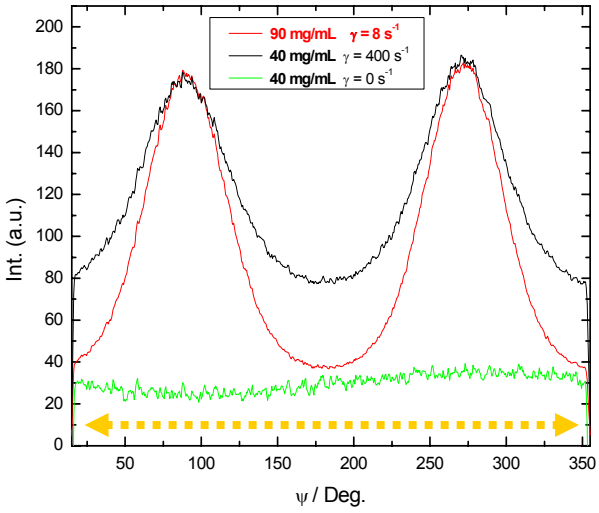

Fig 3 : Azimtuhal profiles taken on SAXS patterns, showing the angular dispersion for a nematic sample (—) and for an isotropic sample undear shearing (—) and at rest (—).

Comparison of the correlation peaks positions (q_{max}) at different concentrations shows that the higher the concentration, the higher the ***q***-value (Cf. Fig.2). Since $\boldsymbol{q_{max}} = 2\pi/\boldsymbol{d_{ave}}$, the helixes interdistance $\boldsymbol{d_{ave}}$ decreases when the concentration increases. Nevertheless, some data suggest that at even higher concentrations (w%=20-30) the q-value decreases again. This would be explained by the spontaneous formation of aggregates due to molecular crowding and attractive interactions overcoming like-charges repulsions. The complete description of this non-monotonous behaviour will require further investigations. Theories of the isotropic-nematic transition in assemblies of rods predict a sudden increase of the order parameter from 0 to 0.8 (0.55 for semi-flexible rods) across the transition. The angular dispersion of SAXS patterns reflects the orientational distribution function of rods in the solution. Fig. 3 shows that a nematic sample as a rather higher order parameter that can be roughly estimated at 0.7. Incidentally, we noticed that shearing of the isotropic phase induces order (Fig.3, black curve), which is also observed in polarized light as transient birefringence when samples are shaken. This shear-induced ordering as been reported for other systems but is still poorly understood at the molecular level.

We have also started to investigate the possible screening effect of salt addition in the solutions in order to describe more precisely the interplay between repulsive and attractive forces. We were also able to induce *in vitro* the spontaneous formation of collagen fibrils, as revealed by the small-angle 67 nm reflexions, characteristic of biological tissues.