Experimental report

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Proposal: 9-13-678 **Council:** 4/2016

Title: The melting of fiber DNA submergedin D-ethanol/D2O mixtures.

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Li-DNA fibers submerged in mixtures of deuterated ethanol and D2O with different ethanol concentrations (7 samples in total)

Instrument	Requested days	Allocated days	From	To
D16	3	3	05/12/2016	08/12/2016
D19	6	6	15/12/2016	22/12/2016

Abstract:

We would like to study the spatial correlations of the DNA molecules submerged in D-ethanol/water mixtures during the melting transition or thermal denaturation of DNA.
Neutron scattering can access spatial information which is fundamental for a total understanding of the transition.
We have performed this kind of experiments with relative success in humidified fibers and fibers immersed in polyethylene glycol (PEG) solutions.
Now we wish to study the melting in ethanol since preliminary measurements have shown a significant difference in the dynamics of the transition with respect to fibers in PEG solution. Namely, the transition seems to be steadier in ethanol/water mixtures which could hint to a different interaction between DNA and solution in these environments. In addition, it is feasible to use deuterated ethanol for composing the solutions so the proton background is lower than in the PEG case and our analysis may be accurate.

The experiment attempted to study the melting transition of DNA fibers submerged in mixtures of heavy water and full deuterated ethanol at different concentrations. The Bragg peak that arises at 1.87 A^{-1} in crystalline samples of DNA is related to the correlation between closed base pairs. Its temperature dependence gives the evolution of the correlation length in the molecules through the transition.
Several samples were studied. All of them consisted of Li-DNA fibers sealed inside aluminum cassettes with solutions of different ethanol content. They were: One ''dry'' sample which was sealed under a 75% humidified atmosphere and six samples with D-ethanol/D_2O mixtures of concentrations 20%, 35%, 50%, 60% and 80% D-ethanol (v/v).
The experiment had two parts. The first one was carried out in D16 and its purpose was to get a structural characterization of the samples as well as to gauge the intermolecular distance in order to quantify the confinement of the molecules. The second one was the melting experiment, performed in D19, in which the evolution of the 10th layer peak in function of temperature was recorded.
Reciprocal space maps (RSM) were recorded at D16. This procedure was repeated for two sample orientations: horizontal (fiber axis in the scattering plane) and vertical (fiber axis perpendicular to the scattering plane). An example of the results can be seen in Fig 1.
The RSM in horizontal orientation (example in Fig. 1a) proved all samples were in crystalline B form (due to the singular broad Bragg peak placed at Q_H =1.87 A^{-1}). In contrast to previous experiments, no signals of A form contamination were found [1], [4].
In previous experiments we have used the RSM in the vertical orientation to detect changes in the distance between molecular axes once the sample were submerged in polymer solutions. For better studying this changes we performed a radial integration and plotted the integrated intensity versus Q (Fig. 2). In the case of the polymer solutions (see [2] and [3]) changes in the crystalline lattices of the samples are apparent in the displacement of the Bragg peaks from one polymer concentration solution to another. In fig 2 the features appear at roughly the same Q for each sample with the exception of the high Q peak of the 35% sample which is found at a lower Q value with respect to its counterparts in the other samples. However, there is a clear change in the structure of the fiber as the ethanol concentration is changed. The peak at 0.32 $Å^{-1}$ is much greater for the 20% and humidified sample than in the other samples. This may be due to a change in the space group caused by the increasing hydration of the fibers.
D19 was used to record the intensity along Q parallel to the fiber axis (Q_H), see Fig. 3. These scans were recorded at temperatures between room temperature and around 100 °C and show Bragg peaks at Q=1.87 reciprocal angstroms which is the reciprocal distance between base pairs. An example of the evolution of the peak is shown in Fig 4. The fitting of this peak at each temperature gives a value for its integrated intensity and width which are central for characterizing the transition. The integrated intensity is proportional to the number of closed base pairs at a given temperature and the width of the peak is inversely related to the average size of the closed domains. The integrated intensity decreased with temperature for each sample and the width increased as the transition was approached (see example in Fig. 5) which is consistent with the sequential opening of the base pairs as the melting proceeds. The evolution in function of temperature of the incoherent background for polymer and ethanol samples is drastically different. Fig. 6 presents the average intensity of the scans of figure 4 in a Q interval in which no coherent contribution is expected (from 0.5 to 1 $Å^{-1}$) in function of reduced temperature for the 60% ethanol sample and for a sample with a solution of water and 17% w/w polyethylene glycol (PEG). The reduced temperature is the real temperature over the melting temperature so at T/T_m= 1 the sample is in the middle point of the transition. As one can see in figure 6 in both samples the incoherent scattering rises as temperature increases which is expected since when base pairs start to break the molecules, which are now some composition of double stranded and single stranded domains, gain freedom of movement and the sample becomes more disordered. In the case of the PEG sample this increase in incoherent scattering starts before the onset of the transition (T/T_m=0.7) and for the ethanol sample it starts almost at the middle point of the transition (T/T_m~1). This suggest a very different effect of the solutions on the DNA fiber.

Figure 1: Reciprocal space maps with the molecular axis in the scattering plane (a) and normal to the scattering plane (b) for the 80% ethanol sample.

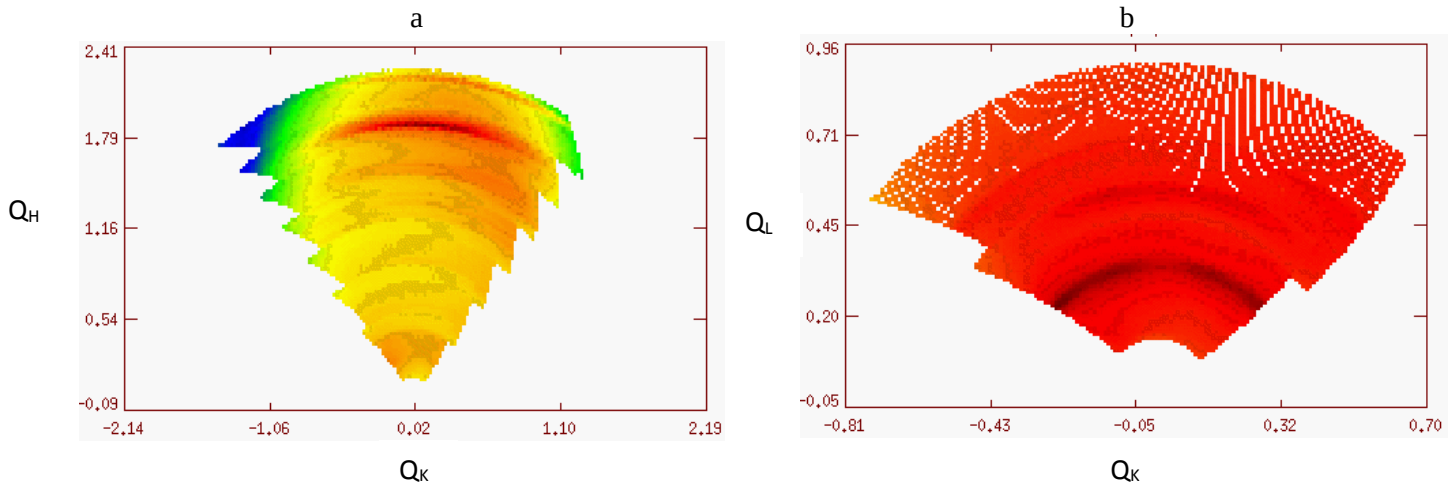


Figure 2: Radial integration of the perpendicular reciprocal space map for each sample.

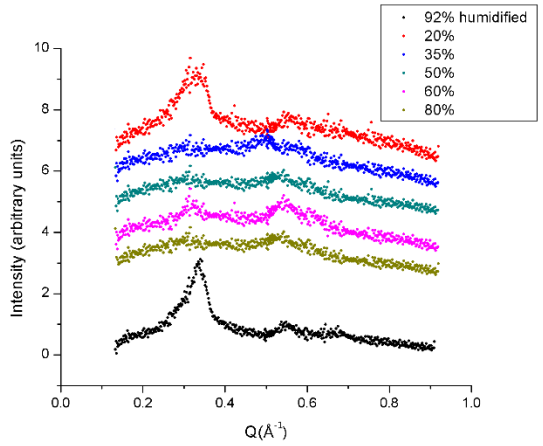


Figure 4: Temperature evolution of the Bragg peak situated at Q_H=1.87 Å^{-1} for the 80% ethanol sample.

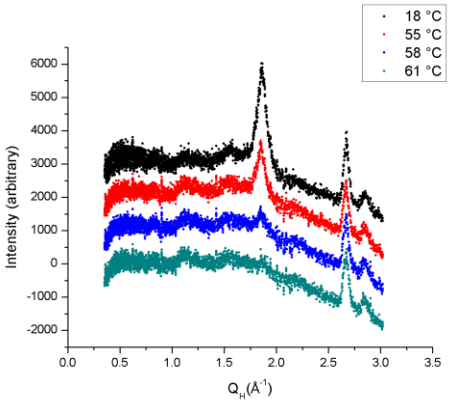


Figure 3: Schematic of the DNA fiber sample inside the aluminum sample holder. The DNA reciprocal coordinate system with respect to the sample cassette has been represented.

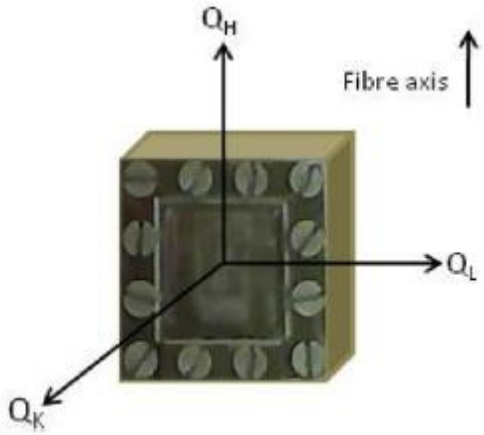


Figure 5: Integrated intensity (black) and width of the Q=1.87 Å peak (blue) in function of temperature as given by our best fit to the date for the 60% ethanol sample.

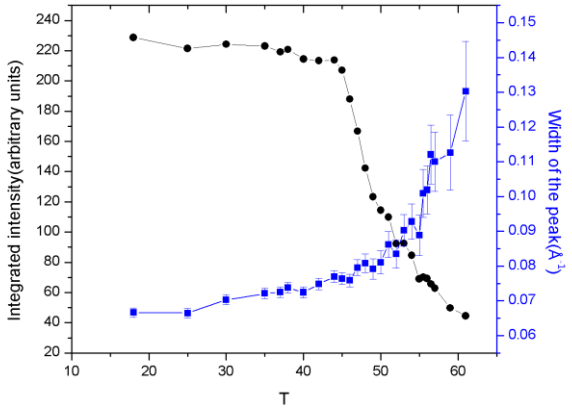
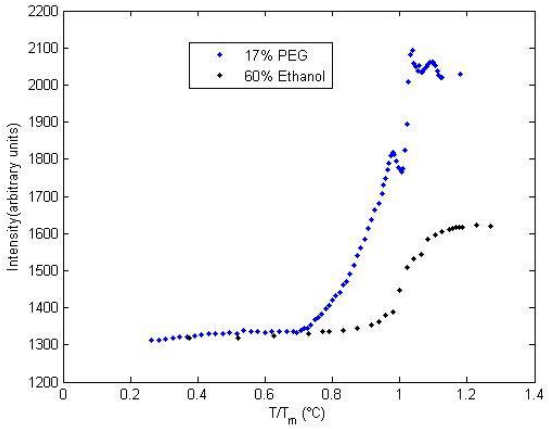


Figure 6: Incoherent background in function of temperature for fibers submerged in D_2O solutions with 60% ethanol (black) and 17% w/w PEG.



REFERENCES

[1]-J. Valle-Orero *et al*, Journal of Physical Chemistry, **117** 1849 (2013).

[2]-Report of the experiment 9-13-625 at ILL.

[3]-A. Wildes *et al*., J. Phys. Chem. B. **119** 4441 (2015) DOI: 10.1021/acs.jpcb.5b01343.

[4]- Report of the experiment 9-13-625 at ILL.