To pump or not to pump: Combining several scattering and optical absorption methods following the formation of biomaterials.

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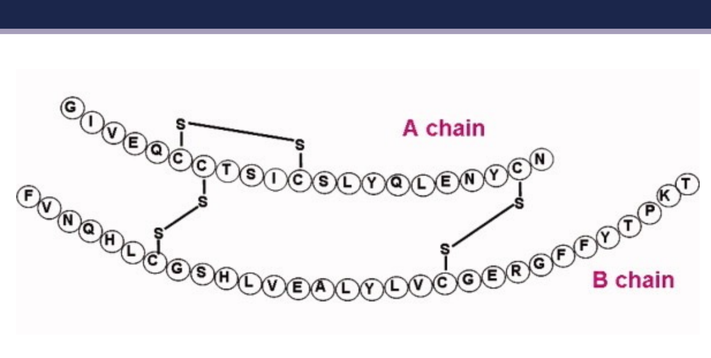
Introduction

We make use of a peristaltic pump to circulate our liquid sample through the cuvettes of different scattering or absorption techniques in order to follow processes in biomaterials formation in time. The tested techniques are UV-Visible spectroscopy, Circular Dichroism, infrared spectroscopy or static light scattering, further more we used small angle x-ray or neutron scattering. As a test sample we investigated the formation of amyloid like structures in insulin at pH 2. Using the amide I band, infrared (IR-) spectroscopy can give information on the fold of the protein and also allows to follow aggregation phenomena. Small angle neutron scattering reports on the global structure of proteins in solution and can give information on the shape of growing aggregates or folded proteins in solution. This is why the two techniques deliver complementary information on the observed process. Since the process of amyloid formation is not very reproducible, the results of different techniques cannot be correlated to each other when they are measured one after the other on different samples. Even if one prepares the sample with great care, the lag phase of the amyloid formation is known to be not very predictable.

Furthermore, we would like to explore the capabilities of infrared spectroscopy based on quantum cascade lasers (QCLs) in combination with other techniques. The advantages of QCLs are superior Gaussian beam characteristics and a higher spectral density as compared to the glow bar infrared light sources of the Fourier-transform infrared spectrometer (FTIR). This allows to measure good quality IR spectra within one second. Their disadvantage is the more complicated pulsed mode of operation and the limited spectral width they can cover.



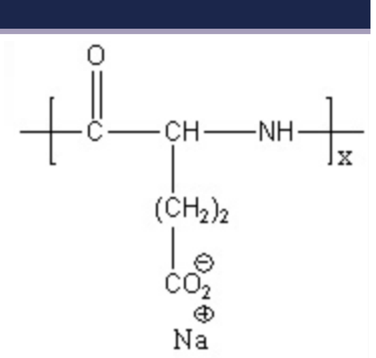


Figure 1: (A) Insulin (Figure taken from [1]) and (B) PLE structures (Figure taken from [2]) respectively.

Amyloid formation in literature

Proteins have an inherent tendency to convert from their native functional states into amyloid aggregates. This phenomenon is associated with a range of increasingly wide spread human diseases like Alzheimer and Parkinson. Amyloid fibrils are thread-like structures typically a few nanometer in diameter and often microns in length. They generally comprise protofilaments that often twist around each other or associate laterally as flat ribbons. The fibrils possess an anti-parallel-β structure, in which β-strands are oriented perpendicularly to the fibril axis and are assembled into β-sheets that run along the longer axis of the fibrils (Figure 2) [4].

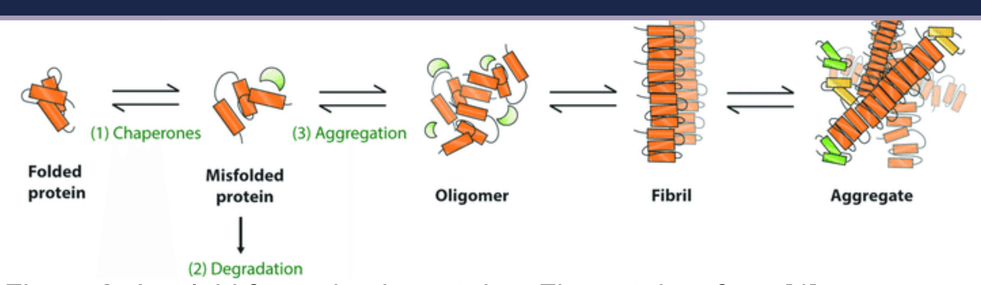


Figure 2: Amyloid formation in proteins. Figure taken from [4].

Results

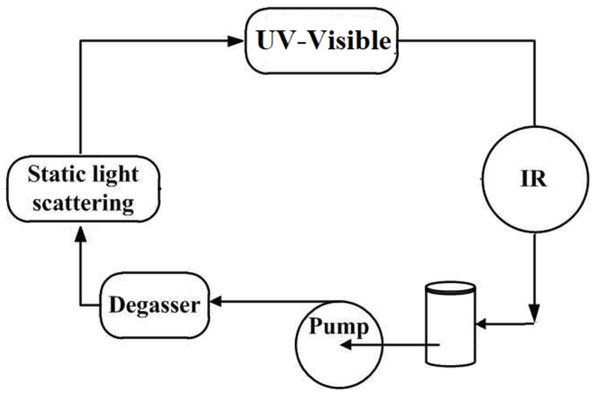


Fig 3A: General schematic for a combination of SLS, IR, and UV-Visible. 20 mg PLE was dissolved in 1 ml acetate buffer then was injected into different machines throw the pump.

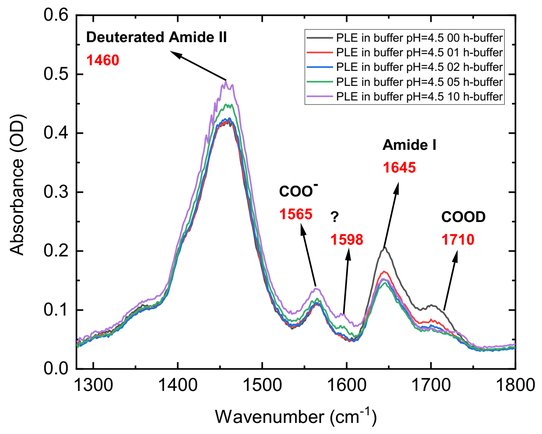


Fig 3B: IR measurement for PLE in acetate buffer. amyloid formation peak can be observed at 1598 cm^{-1} .

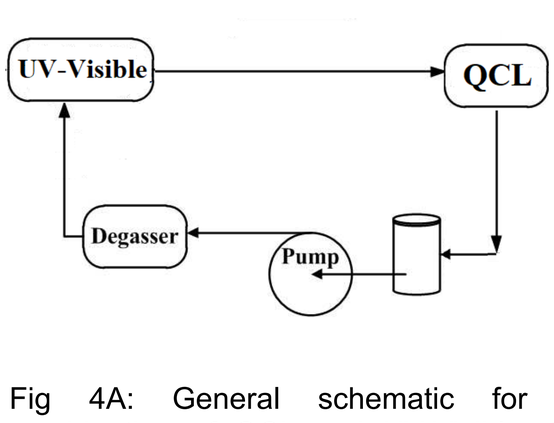


Fig 4A: General schematic for a combination of QCL and UV-Visible. 20 mg insulin was dissolved in 1 ml phosphate buffer then was injected into different machines throw the pump.

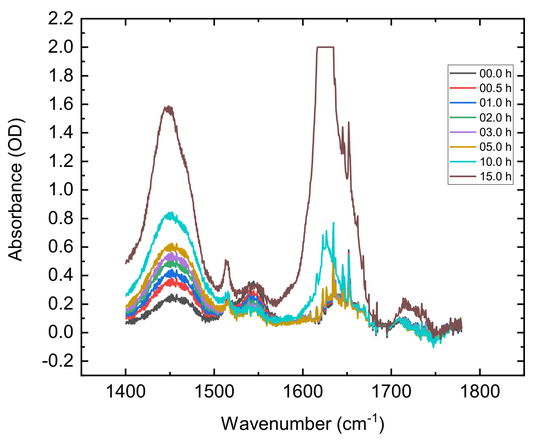


Fig 4B: QCL measurement for insulin in phosphate buffer. amyloid formation peak can be observed at 1627 cm^{-1} .

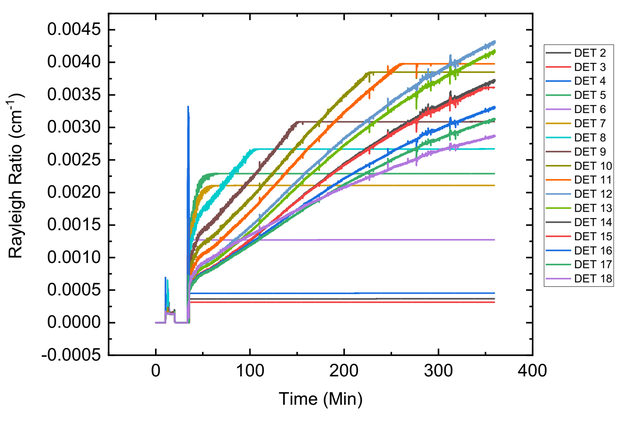


Fig 3C: SLS measurement for PLE in acetate buffer. With increase in time there is no increase in rayleigh ratio which could be due to saturation in PLE solution.

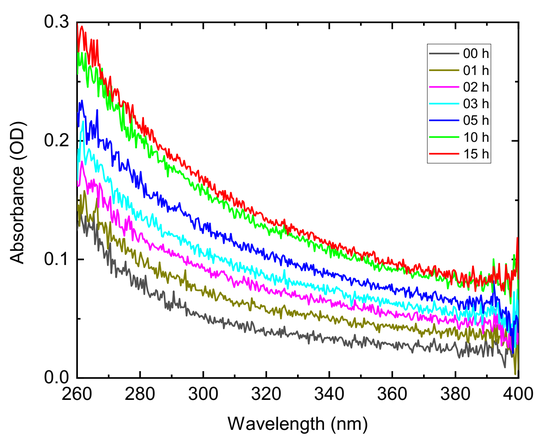


Fig 3D: UV-visible measurement for PLE in acetate buffer. Absorbance as a function of time increased with increase in time. At a certain time at lower wavelengths the absorbance was higher.

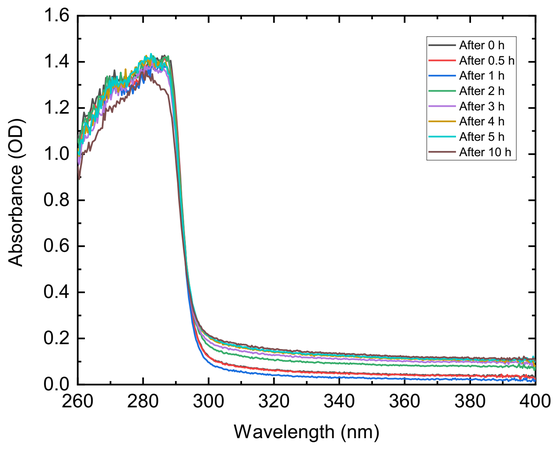


Fig 4C: UV-visible measurement for insulin in phosphate buffer. At lower wavelengths (260-280 nm) the absorbance is much higher. Also, over time, absorbance for insulin increased.

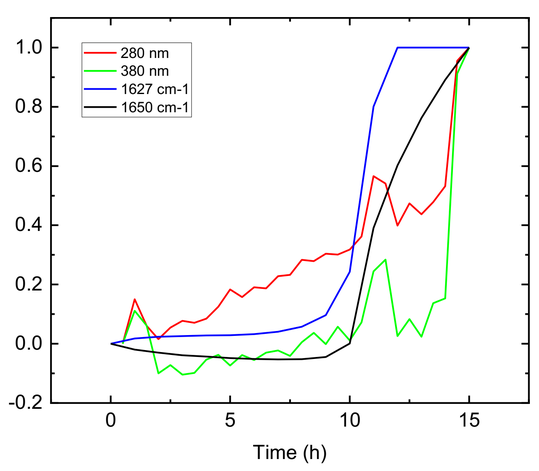


Fig 4D: Spectra for UV-visible and QCL after normalization. The same results were obtained from both techniques which confirm amyloid formation in insulin in phosphate buffer at pH=2.

References

1. Mayer, J. P., Zhang, F., & DiMarchi, R. D. (2007). Insulin structure and function. Peptide Science: Original Research on Biomolecules, 88(5), 687-713
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