

Effect of the hop extract and of bioactive compounds from hop plant on the aggregation of human insulin into amyloid fibrils

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INTRODUCTION

Protein aggregation into amyloid fibrils is a phenomenon attracting attention from a wide scientific community, mainly because the presence of mature fibrils is linked to several neurodegenerative diseases [1].

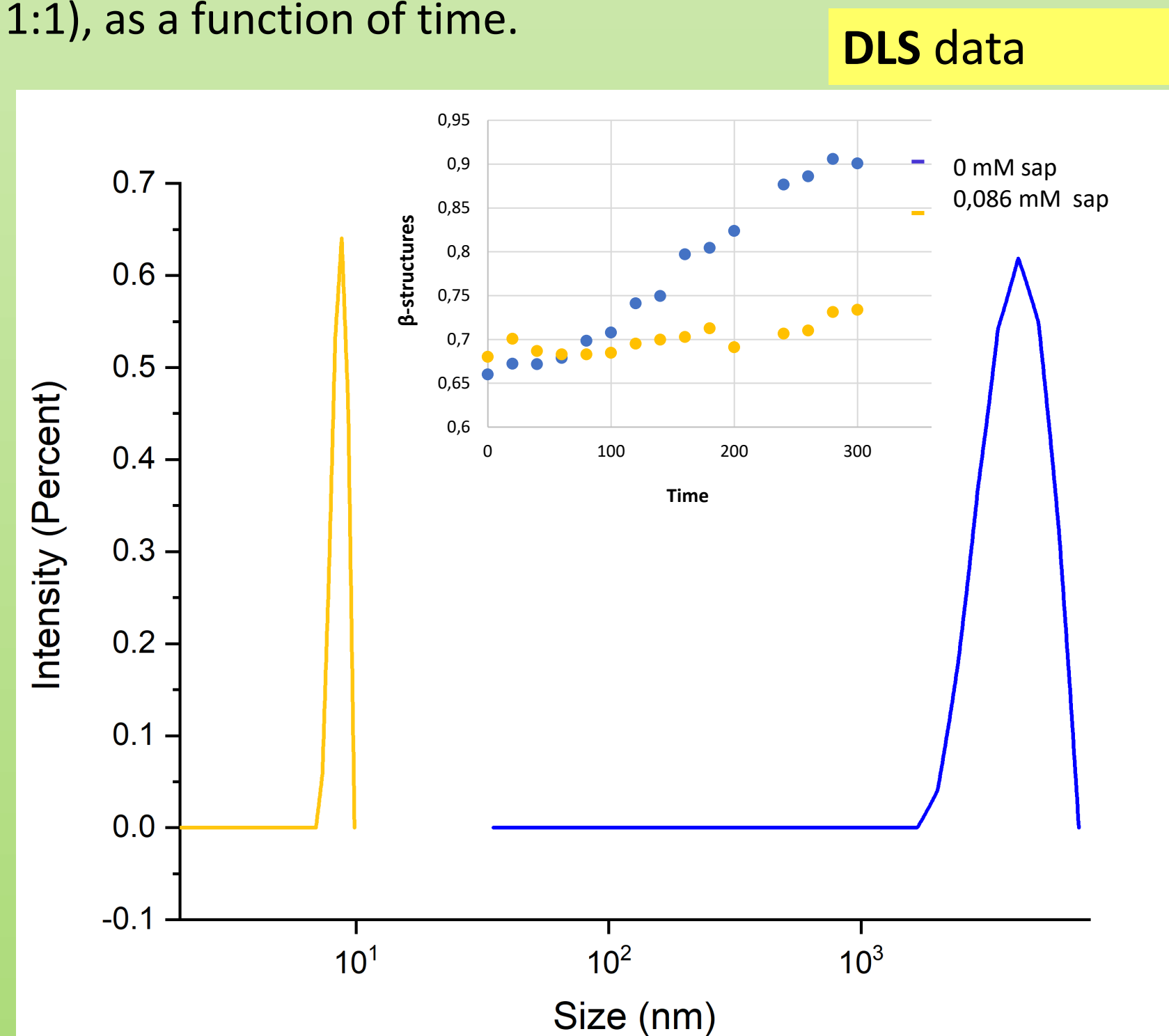
Since it is known that some natural compounds from plants can maintain protein-functionality in denaturing conditions, for this work we used saponins and hop extracts (from plant waste) to test their influence on human insulin fibrillation. Recently, beneficial effects of hop extracts have been shown for amyloid beta peptide aggregation, related to Alzheimer's disease, too [2].

To monitor the kinetic of amyloid aggregation we used UV-Visible absorption and fluorescence spectroscopy, using Congo Red and Thioflavin T as amyloid specific probes [3]. To monitor any secondary structure changes (from α -helix to β -sheets) we performed Circular Dichroism experiments, and to obtain the overall structural features and size of aggregates we performed Synchrotron Small Angle X-ray Scattering (SAXS) and Dynamic Light Scattering experiments (DLS).



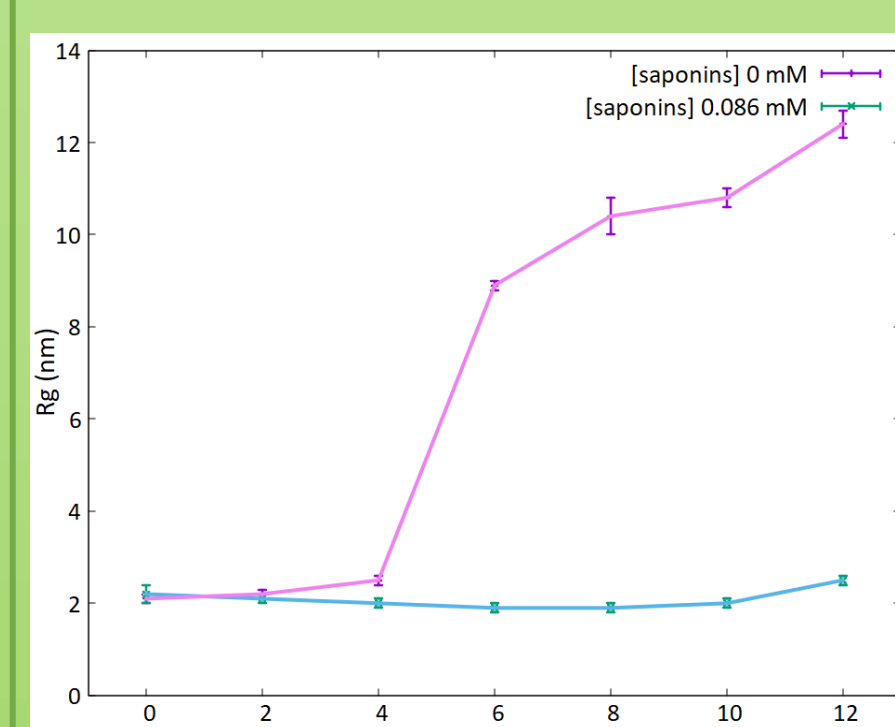
EXPERIMENTS WITH SAPONINS

All data are obtained for human insulin in phosphate buffer 50 mM, pH 7.4, at 37° C in gentle agitation, in absence and presence of saponins (molar ratio insulin-saponins 1:1), as a function of time.

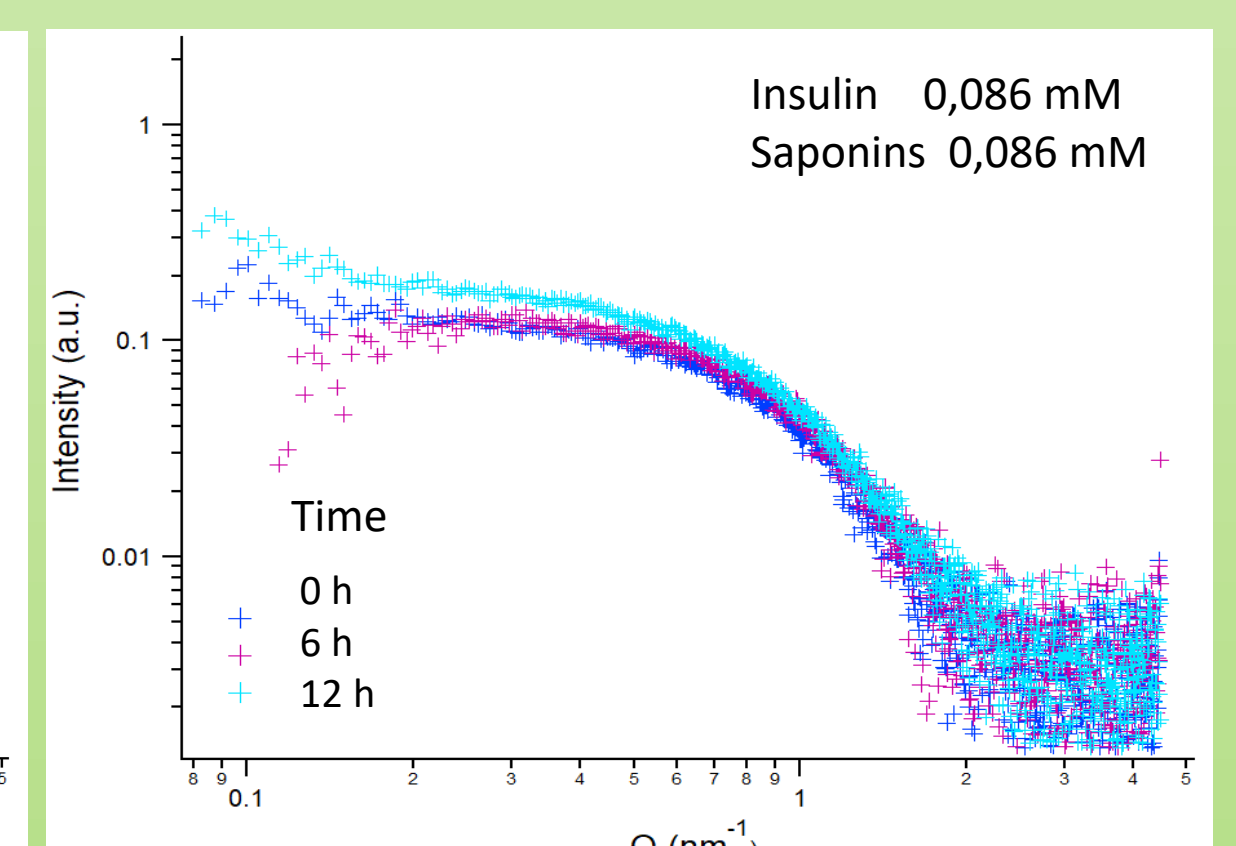
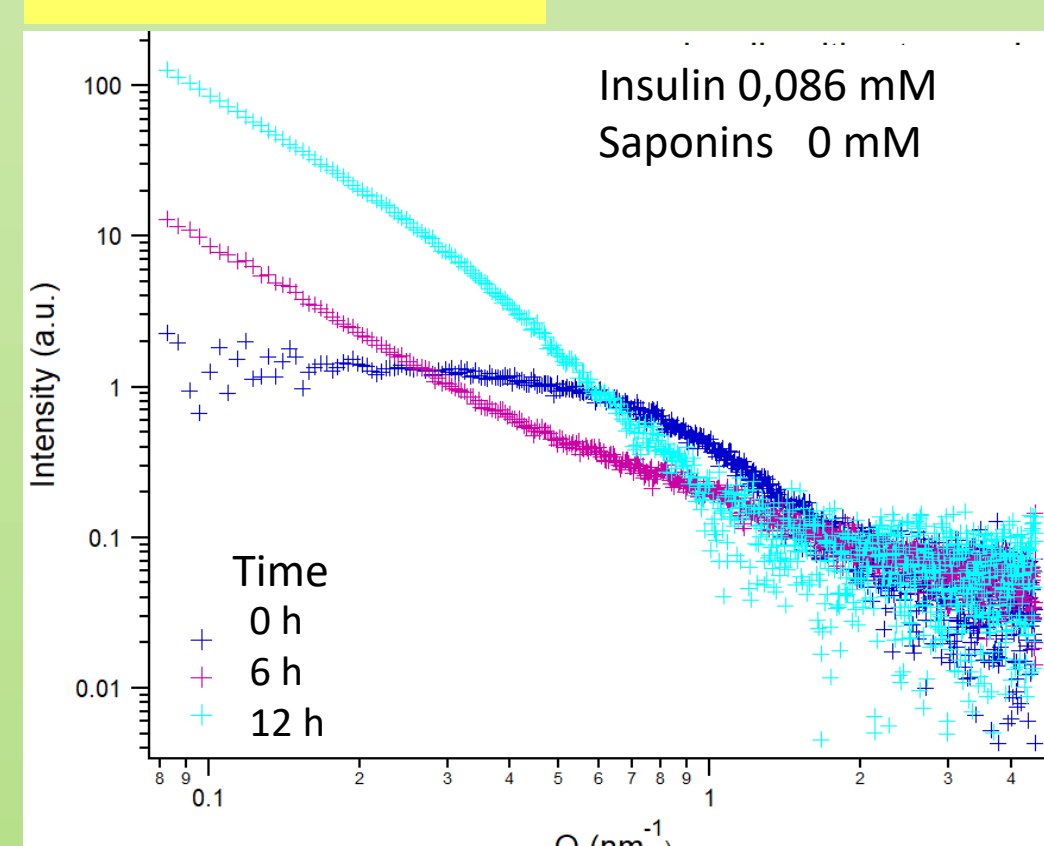


Size distribution obtained by DLS for the final state of human insulin kinetics. In the inset, absorption spectroscopy results.

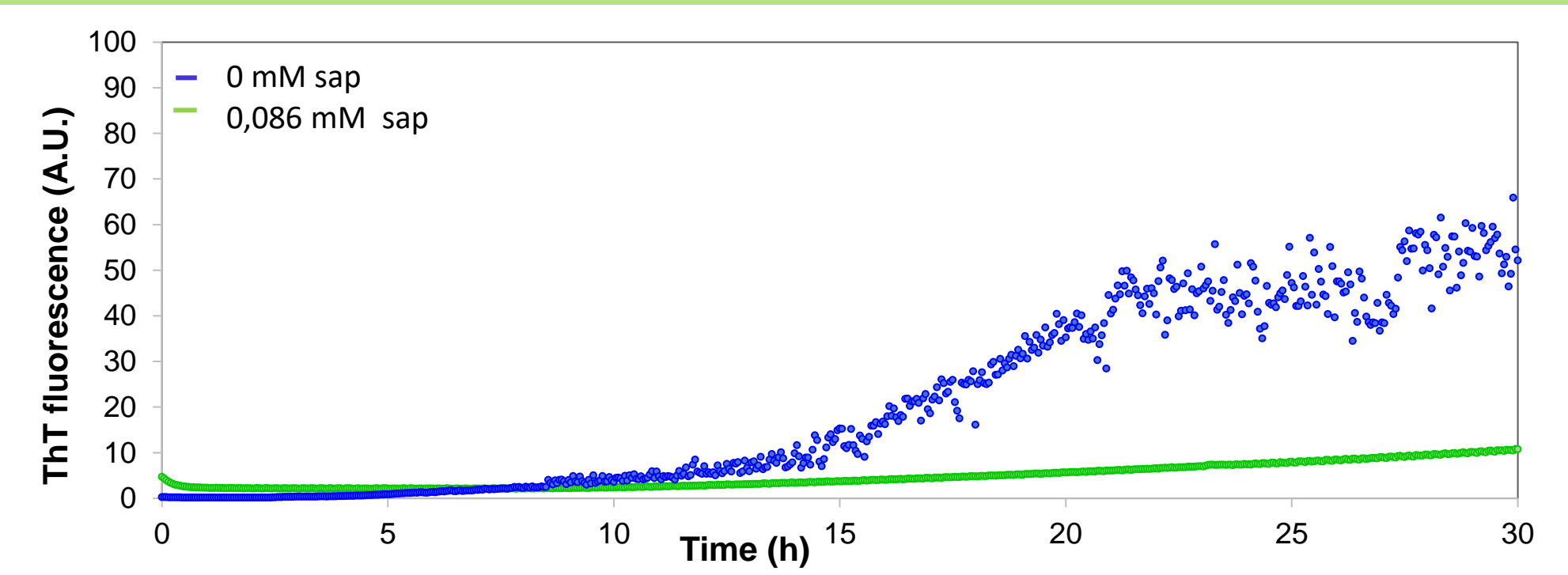
The radii (R_g , R_c) obtained with Guinier and Guinier rod-like analysis confirm the inhibiting effect of saponins on aggregation



SAXS data



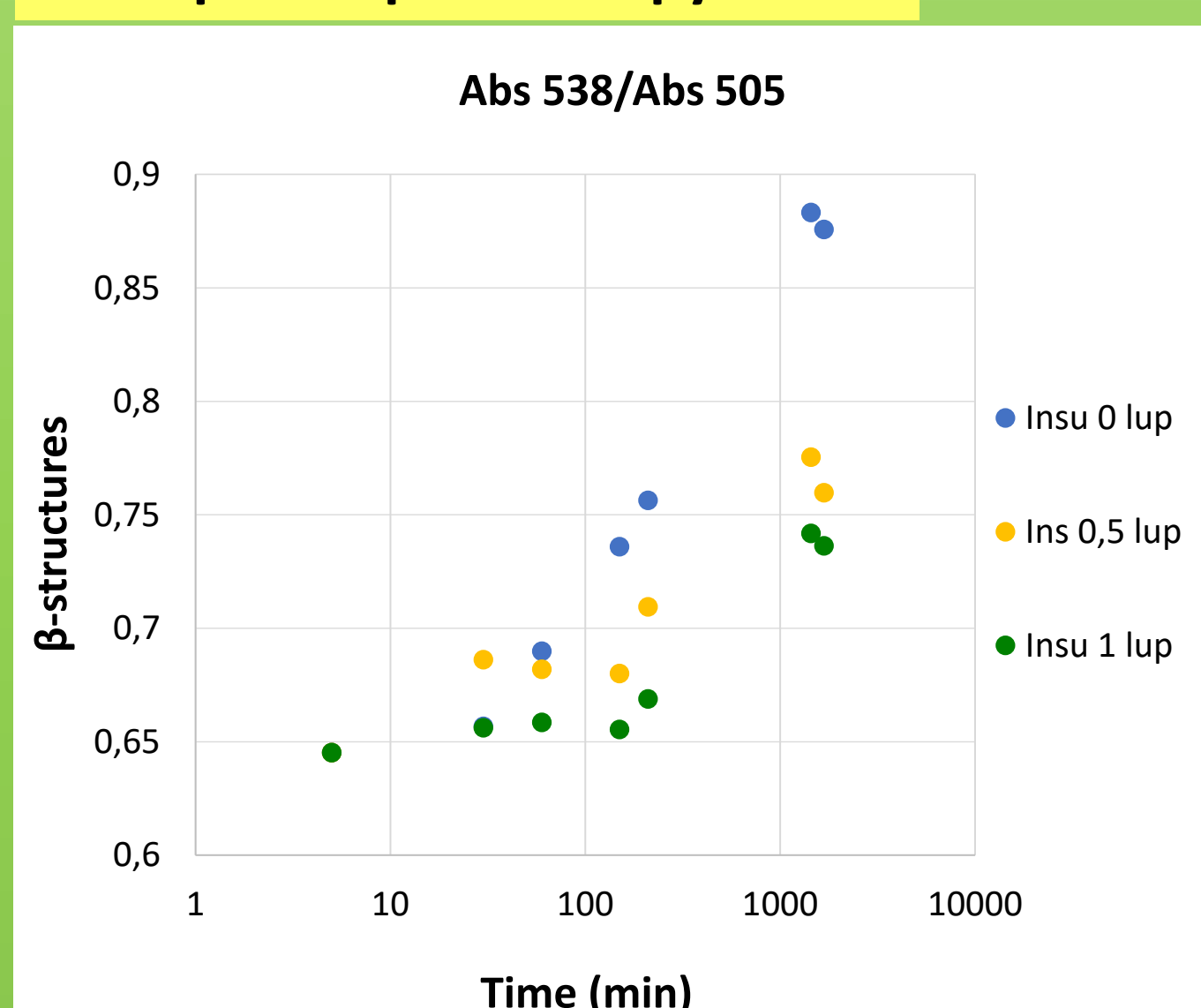
ThT fluorescence data



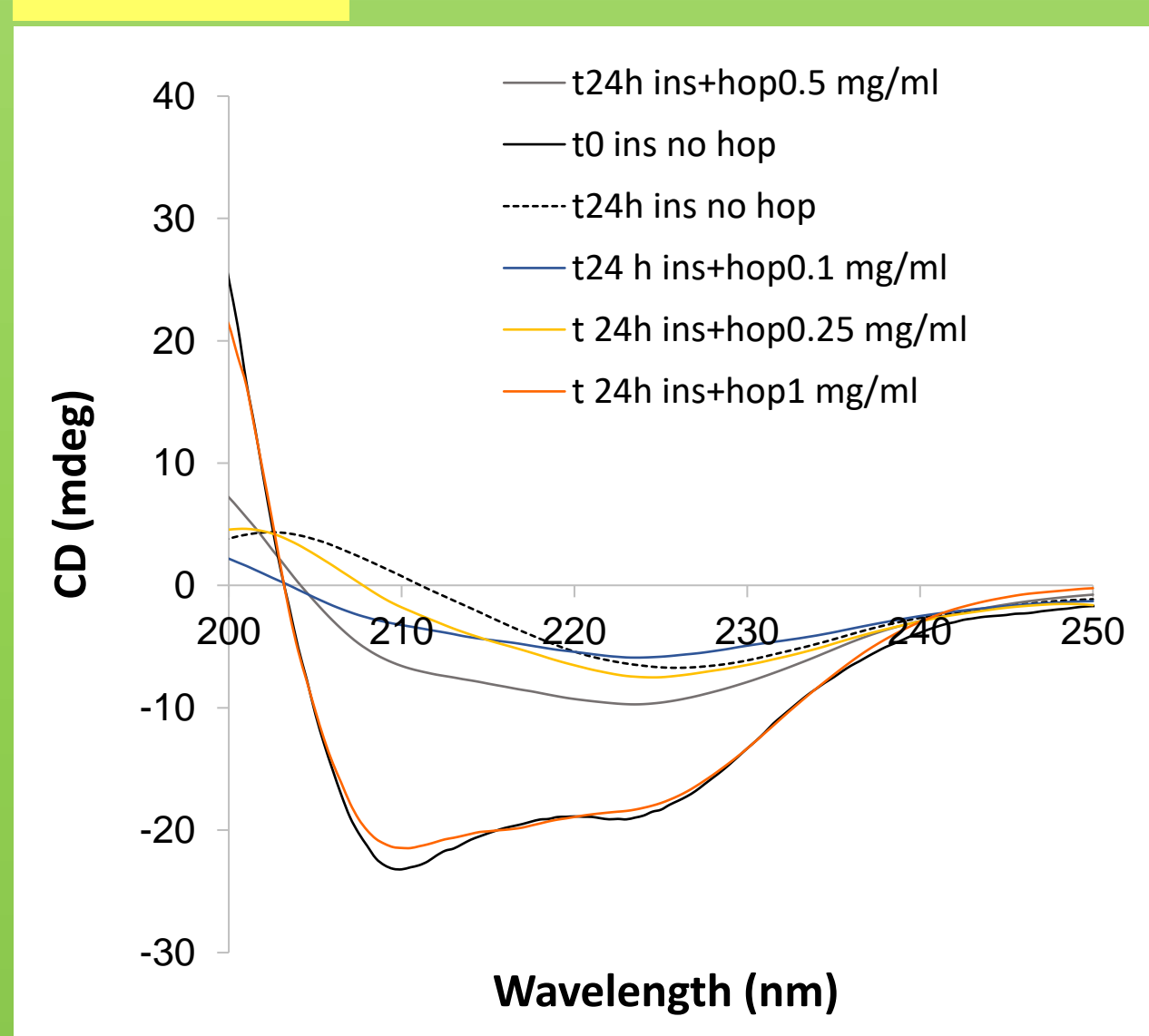
EXPERIMENTS WITH HOP EXTRACT

All data are obtained for human insulin in phosphate buffer 50 mM, pH 7.4, at 37° C in gentle agitation, in absence and presence of the hop extract (at different concentrations), as a function of time.

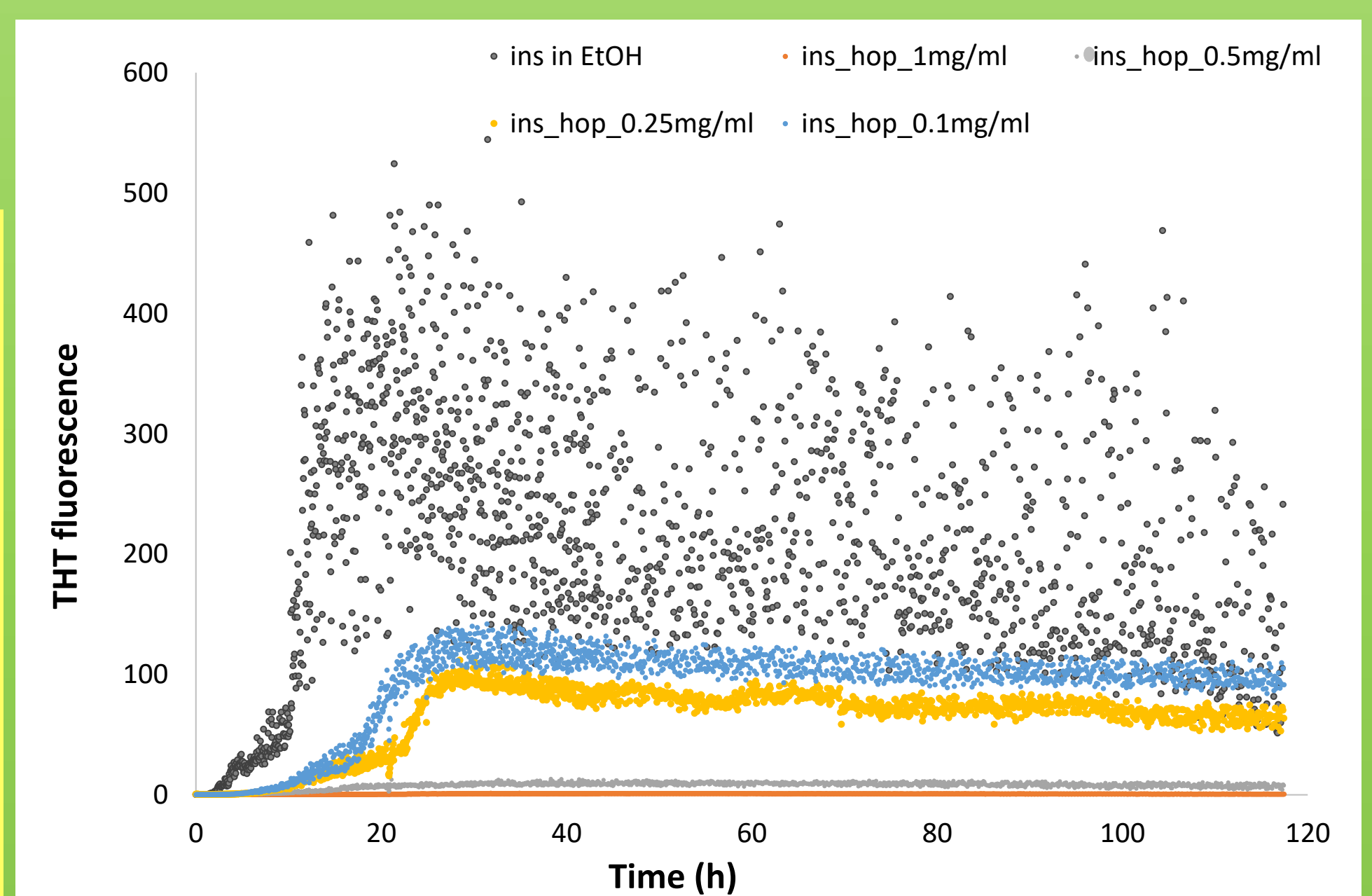
Absorption Spectroscopy data



CD data



ThT fluorescence data



CONCLUSIONS AND PERSPECTIVE

Our experimental results suggest that the bioactive components of hops interfere with the amyloid aggregation:

- saponins have been shown to induce a high inhibition on the formation of insulin fibrils;
- also our first data for the whole hop extract show that the extract produces a slowing down of the kinetics and a reduction in the final number of fibrils.

We are going to perform further aggregation experiments with other bioactive compounds from hop plant.

The biomass of hops, in this case extracted from the hop leaves, can be a precious resource, having several application potentialities, for the pharmaceutical and nutraceutical industries. Because the hop extracts are obtained from the agricultural waste, this investigation consequently aims to pursue the proposal of circular economy model.