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SCIENCES ET TECHNOLOGIES POUR L'ÉNERGIE ET L'ENVIRONNEMENT

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Object : report on the dissertation of Ing. Monika Holubová

Dear Chair of the Committee,

Please find below my report on the dissertation of Ing. Monika Holubová.

The thesis of Ing. Monika Holubová deals with the investigation of the effect of two types of organic materials, i. e. carbon nanoparticles and polymers, on the aggregation of amyloids.

The thesis is well written, (in English), synthetic despite the large number of experimental outcomes and very pleasant to read. It is divided in two parts, being the first one devoted to the state of the art and focusing particularly on amyloidogenic peptide/proteins and the associated issues with amyloids aggregation. This first part further gives an overview of nanomaterials prior to a description of the main methods Ing. Monika Holubová used to conduct her investigations. The second part of the dissertation is divided into two sections. The first one describes the interaction of amyloids with carbon nanospecies whereas the second one explains the effect of polysaccharides on amyloids aggregation.

The first part of the thesis positions the activities of Ing. Monika Holubová regarding the state of the art and clearly shows that even if not all amyloids are harmful, many are associated to diseases named amyloidoses. The studies conducted by Ing. Monika Holubová are thus of high interest since the outcomes of her work might evidence the role of some materials, such as carbon nanospieces and biological polymers in the onset of the amyloidoses. The first part of the state of the art gives a comprehensive description of amyloids, their function and their identification in human pathologies. Ing. Monika Holubová has not omitted to recall that some systemic forms of amyloidoses may be secondary (resulting from another disease or hereditary) such as those related to the (Apo) serum amyloid A or to transthyretin. The first part of this chapter describes as well the mechanism of amyloids aggregation and the conventional approaches to follow the process of amyloids genesis over time (thioflavin T fluorescence spectroscopy).

Material used in nanotechnology are clearly described and classified according to their size and shape in the subsequent section of this first section. The methods used for the

preparation of nanomaterials are presented as well. Ing. Monika Holubová clearly describes according to which potential pathways they may endanger human health.

Ing. Monika Holubová then describe the methods she used to characterize the system she investigated. The effect of both carbon nanospecies and of polysaccharides and glycogen modifications on amyloids aggregation were investigated with the hen egg white lysozyme (HEWL) as well as with the amyloid β (1-42) ($A\beta_{1-42}$) model systems. She resorted with success to transmission electron microscopy (TEM) imaging in combination with other techniques, in particular to ThT fluorescence spectroscopy.

The second part of the dissertation is devoted to the results and their discussion. Ing. Monika Holubová focused on the evaluation of the influence of carbon nanospieces and polysaccharides on the process of amyloids aggregation.

Since these nanomaterials are produced by both advanced methods but also by common processes such as burning and pyrolysis of organic materials, arc discharge welding and explosion, she has chosen to focus on fullerenes (C_{60}) carbon quantum dots (CDs), single walled carbon nanotubes (SWCTs) and nanodiamonds (NDs).

She combined UV-vis absorption spectroscopy, elemental analysis, Fourier Transform Infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS) and nuclear magnetic resonance (NMR) to comprehensively characterize CDs. SWCT, C_{60} and NDs were as well characterized by dynamic light scattering (DLS) and TEM. Based on the experimental results, Ing. Monika Holubová proposes a mechanism of SWNT inhibition of HEWL amyloids aggregation.

In a further step, Ing. Monika Holubová determined the influence of polysaccharides, i. e. glycogen (GG), phytoglycogen (PG) and mannan (MAN) and several glycogen modifications on the process of amyloids aggregation. She conducted the alkylation of GG through the reaction of its alkoxide with benzoyl or cinnamoyl chloride to produce the desired modified glycogen. The polymers used in this part were characterized by asymmetric flow field-flow fractionation (AFFFF). ThT fluorescence spectroscopy was mainly combined with TEM to investigate the effect of polysaccharides on HEWL amyloids aggregation. Interestingly, temperature dependent measurements were conducted and mainly monitored by DLS.

Overall, Ing. Monika Holubová demonstrated, as reported in her dissertation that the process of amyloids aggregation depends on the size, shape and surface properties of the nanomaterials. She evidenced that aggregation is influenced by the affinity of the protein with the nanospecies surface properties.

She assessed the following trend regarding the induction of amyloids aggregation with CNPs: NDs>control>C60>CDs>SWNTs.

HEWL fibril formation was promoted with increasing concentrations of PG and GG whereas MAN had very little effect. Noticeably MAN did affect $A\beta_{1-42}$ readily formed fibers. Physiologically relevant concentrations of GG tremendously accelerated HEWL aggregation. PG and GG shortened the lag phase and slightly accelerated the growth phase of $A\beta_{1-42}$. Slight changes in the polysaccharide surface might accelerate or retard amyloids aggregation.

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The original outcomes reported in this dissertation are strongly supported by experimental outcomes. It is a very competitive field of activities and I truly appreciate the different approach Ing. Monika Holubováhas has chosen to tackle amyloids aggregation: she does not look at the identification of a therapeutic but rather investigate the effect of materials localized in the body, whether they are naturally present or not, on the amyloids aggregation process. The activities of Ing. Monika Holubová are highly relevant as acknowledged by five publications as first author in peer reviewed journals and by a book chapter. She took part to two conferences in Czech Republic and to one in Slovaquia. Based on this report I strongly recommend the defense of this PhD thesis. I would on that day discuss further the following points with Ing. Monika Holubová.

- Why did she focused on amyloids aggregation although biochemical routes have been evolved by Nature to induce disaggregation of readily formed fibers? Which are these natural routes?

- How in vitro amyloids aggregation compares with in vivo, physiological conditions?

- Temperature dependent measurements were conducted when investigating the interactions of polysaccharides with amyloids? How could the outcomes be extended to the interaction of carbon nanoparticles with amyloids if it were possible?

- Ing. Monika Holubová mentioned in the state of the art that some amyloids are functional whereas others are pathogenic. Why did she select two pathogenic models of amyloids aggregation? Which model of functional amyloids could she chose and why?

- Ing. Monika Holubová describes a possible mechanism of SWNT interaction with HEWL, as schematically shown on Figure 20. Why such a possible mechanism has not been proposed to describe the interaction of the other carbon nanomaterials and polysaccharides with amyloids? On the basis of the outcomes of the interaction of polysaccharides with $A\beta_{1-42}$ what possible effect could be expected on the interaction of carbon nanomaterials with $A\beta_{1-42}$

Should there be any further questions regarding this report, please do not hesitate to get in touch.

Faithfully,

Corinne Nardin

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