INSTITUTE OF MACROMOLECULAR CHEMISTRY
CZECH ACADEMY OF SCIENCES

Department of Chemistry and Physics of
Surfaces and Biointerfaces
Heyrovského nám. 2,
162 00 Prague 6,
Czech Republic
www.imc.cas.cz

Evaluation of Ph.D. dissertation thesis submitted by Ing. Pavel Vostrejš

The dissertation thesis submitted by Ing. Pavel Vostrejš focuses on isolating lignin from agricultural wastes using the soda pulping method, with particular emphasis on achieving high antioxidant activity. The thesis focuses on utilizing various lignin sources (Kraft lignin, organosolv lignin, grape seeds soda lignin, plum sheels soda lignin, etc.). Among all, rape seed lignin exhibited the highest antioxidant properties and was subsequently used to modify polyhydroxyalkanoate (PHA) cryogels enchancing their thermal stability and antioxidant capacity. The lignin samples subjected to biotechnological modification using white-rot fungi showed improvement in antioxidant properties albeit the unexpected changes in molar mass of the polymer. The thesis also focusses on the preparation of lignin nanoparticles (LNP) suggesting aqueous tetrahydrofuran (THF) solution to be the most effective in the formation process. The LNPs in combination with various polycations (polyallylamine hydrochloride (PAH), poly-L-lysine (PLL), polydiallyldimethylammonium chloride (PDM) and polyethyleneimine (PEI)) were used for the *in-situ* layer-by-layer (LbL) build-up of surface confluent films. The surface modification was investigated by quartz crystal microbalance with dissipation analysis (QCM-D). The highest LNP adsorption was achieved using poly-L-lysine, forming films exhibiting both antioxidant and antimicrobial functionalities. Finally, the thesis concludes to demonstrate that lignin isolated from agricultural residues can be processed and further modified, highlighting its potential for integration into sustainable, high-performance materials.

The dissertation mainly deals with the careful understanding, optimization, challenges, downfalls, fragmental knowledge and utilization of lignins of various origin for the design of PHA cryogels of increased thermal stability and antioxidant capacity. Particular focus is set on the physico-chemical characterization of the lignin chemical/covalent structure, solubility, antioxidant activity, molar mass, etc. and the employment of lignin for the formulation of LNP's and the *in-situ* monitoring of LbL build-up using QCM-D.

The thesis consists of Doctoral Thesis Assignment section, Abstract, Keywords, Declaration, Acknowledgement, Referenced content, Introduction, Theoretical Section, Aims, Experimental section summarizing the utilized materials and methods, synthesis and biotechnological modifications procedures, biological sources, microorganisms tested, equipment, etc., results and discussion part summarizing the obtained main results, conclusions, resume written in Slovak language and referenced bibliography. The submitted thesis has 141 pages. The work is well concepted and organized with a clear presentation and vision. The introduction provides a very careful resume on the developments in the field. The candidate addresses and explains all obtained results independently going well beyond his already published works. I highly value the fact that every abbreviation is stated in the provided abbreviation list. At the end of the dissertation the candidate's Curriculum vitae is provided, stating the academic awards, published scientific papers on which the dissertation builds and the conference contributions.

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Despite the fact that the work is rather precisely written, I have to point out few discrepancies, typos, inconsistencies and redundancy:

i) Utilization of molecular weight of polymers throughout the thesis, while IUPAC recommends molar mass;
ii) Physical quantities E^*, E', E'', M_w, M_n etc. often used in non-cursive throughout the whole thesis, often with omitted use of subscripts for "weight" or "number" averages;
iii) Page 43, missing bracket equation (2);
iv) Redundant data Figure 26 and Table 10; one could report only the figure or the table;
v) Page 46, PHA "gryogels";
vi) Page 67, "doble";
vii) Page 78, non-defined abbreviation "nd" meaning "not demonstrate" but can be referred to also as "not determined", etc.

However, the pointed-out inconsistencies or typos are minor and do not seriously affect the quality of the dissertation. The thesis mainly builds on the first-authored publications which have been published in journals of high scientific visibility. In my opinion the applicant has shown a high level of independence, diligence and creativity, undoubtedly thus demonstrating to be fully capable of performing independent research.

Therefore, I **recommend Ing. Pavel Vostrejš´s dissertation for defense at the Institute of Food Science and Biotechnology, Faculty of Chemistry, Brno University of Technology (Brno, Czech Republic)**.

Specific questions to the dissertation:

1) The thesis relies on the FTIR characterization of lignin samples of various origins and well as on following the modification of lignin samples. Did the applicant perform some deconvolution using "reference material" spectra's to reliably confirm their presence and probe their possible contributions? Did the Author consider correlating the FTIR findings with the performed NMR analysis?
2) Within the solubility analysis and the observed complete solubility of alkali lignin (Page 60) the Author discusses possible residual alkali compounds. This contradicts the Elemental composition reported in Table 10. Can the Author give his critical opinion on this issue?
3) The Author analyses the surface wettability of the porous materials using the sessile drop method (Page 72, Section 5.3.6). This can be problematic as the drop can protrude through the porous sample. Can the Author give his critical opinion on the measurement and observed wettability/hydrophobicity?
4) I as a Reviewer could not follow the motivation behind the studies on lignin NP formation. Can the Author in detail address the motivation for the study of NP formation?

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5) DSC analysis demonstrated the existence of crystallites. Did the author perform some additional analysis to probe the crystallinity and it's content within the material?
6) The author follows the formation of LbL films between LNP's and various polycations. What was the molar mass of used polycations? To which extent the observed capacity of poly-L-lysine is affected by the M_n of the polymer?
7) The Author monitors the formation of LbL films *in situ* via QCM-D analysis. However, the dissertation reports only the third overtone and does not consider the response of higher overtones. Did you attempt analyzing the higher overtones and probing the data set beyond the Sauerbrey equation? As you suggest viscoelasticity of the LbL films, can you elaborate the possible viscoelastic modeling of the data?
8) Why did you use the LNP's for monitoring the LbL film formation instead of using the non-assembled lignin polymer?

With kind regards,

Dr. Ognen Pop-Georgievski
Department of Chemistry and Physics of Surfaces and Biointerfaces
Institute of Macromolecular Chemistry, Czech Academy of Sciences
Heyrovského nám. 2,
162 00 Prague 6,
Czech Republic
www.imc.cas.cz