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Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Faculty	Faculty of Science
Procedure field	Analytical Chemistry
Applicant	**Mgr. Tomáš Vaculovič, Ph.D.**
Applicant's home unit, institution	Department of Chemistry
Habilitation thesis	**Laser ablation as a powerful tool for determination of elemental distribution**
Reviewer	PECHEYRAN Christophe, PhD, Habilitation obtained in 2014
Reviewer's home unit, institution	Institut des Sciences Analytiques et Physicochimiques pour l'Environnement et les Matériaux (IPREM), Université de Pau et des Pays de l'Adour-CNRS, 2 Avenue Pierre Angot, 64053 Pau cedex 09, France

[Review text]

Dr. Tomáš Vaculovič presents a manuscript entitled "Laser Ablation as a powerful tool for the determination of elemental distribution" for his Research Habilitation defense.
My general impression of this document is that it is very well written, in a simple, concise and didactic form.

In a brief introduction, Dr. Vaculovič presents the scientific background and the growing interest in understanding the spatial distribution of chemical elements, one of the tools of which may be laser ablation -ICPMS. The structure of the manuscript is clearly explained from the beginning: it is a summary of 16 high ranking publications of which Dr Vaculovik is co-author, selected on the theme of the analysis of the spatial distribution of chemical elements by LA-ICPMS. I particularly appreciated the effort that has been made to specify the involvement of the candidate for each of them in the different tasks that contributed to the publication process (Experimental work, Supervision, Manuscript writing, research direction).

It should be noted that Dr. Vaculovič is already an internationally recognized researcher. He is the author of more than 60 peer-reviewed papers published in a wide variety of research fields divided according to Scopus as follows: Chemistry 29, Earth and Planetary Sciences 22, Materials Science 9, Physics and Astronomy 9, Biochemistry, Genetics and Molecular Biology 7, Engineering 5, Environmental Science 4, Arts and Humanities 3, Chemical Engineering 3. This attests to his dynamism, open-mindedness and key role he plays in analytical chemistry at Mazaryk University. Again according to Scopus, his H factor is of 12. This index should rapidly increase due to his impressive publication rate in the last years (10 papers in 2019 and already 8 for 2020). Participation to international conferences as regular or invited speaker is however missing in the manuscript as it is difficult to obtained via

internet. However, this information would only be a further illustration of the scientific weight of Dr. Vaculovič and the fact that it is missing from the manuscript is in no way detrimental.

In a second part, Dr. Vaculovič provides a background on laser ablation analysis, a description of laser operation and a brief summary of instrumental developments that have been made since 1960. He reports the importance of wavelength and pulse duration on the particle size distribution and on the selective evaporation of elements (and thus on analytical accuracy). It then describes in more detail different ways of compensating for the variation in the ablation rate that occurs from one material to another (depending on their mechanical, optical or thermal properties, or due to a variation in the energy delivered by the laser over time). Advantages, limits and applicability of internal normalization, normalization to the total sum of oxydes, normalization to the sum of ion intensity and total mass removal are then presented.

Then the third and last part of the manuscript reports on the contribution of Dr. Vaculovič in LA-ICPMS imaging on three scientific application domains: corrosion of metallic samples, geology and bio-applications.
Corrosion of metallic samples deals with a case study of the aging/corrosion processes of new materials for nuclear reactors when exposed to molten fluorides salts used for cooling. 4 papers are summarized in this section. LA-ICPMS is used here to identify the thickness of the corrosion layers, corrosion damages and elemental changes in structural material in a qualitative and quantitative approach. EPMA imaging is systematically compared to LA-ICPMS showing good agreements.
Geological applications section refers to 5 papers. Among them, paper 8 ("Quantification of elemental mapping of heterogeneous geological sample by laser ablation inductively coupled plasma mass spectrometry, Vaculovič et al, 2017) appears as one of the most important for the candidate as he developed innovative quantitative imaging methodology, ie total sum of oxides compared to internal standardization.
The last subsection "Lateral distribution for bio-applications" definitely represent one of major field of interest of Dr. Vaculovič. 7 innovative papers are presented here. Dr. Vaculovič first reports an interesting study published in Applied surface Science (Vaculovič et al 2015) on the influence of laser ablation parameters on trueness of imaging. Then Zn and Cu distribution in melanoma tissue have been studied showing accumulation of these elements in the growing melanoma tissues. In a next study cancer treatment via xenobiotics as Pt-based cytostatics have been considered showing different Pt penetration behavior and release into the tumor tissues when selectively oxidized cellulose is used as Pt carrier, compared to Cis-Pt. This represents new hope in cancer treatment.
LA-ICPMS molecular imaging using gold nanoparticles bioconjugates and more particularly with molecular imprinted polymer is an important part of the manuscript as it brings, together with other studies published by several authors, the new trend of LA-ICPMS for biomedical applications. This was applied by Dr Vaculovič to metallothionein detection, and IgG.

Finally, a brief conclusion is provided giving 2 directions for the future of LA-ICPMS. The first one emphasizes the need to shorten the analysis time as well as improving the spatial resolution are the next challenges to be addressed. The second, emphasizes on the use of

biorecognition for the determination of proteins, making LA-ICPMS a new type of “molecular microscope”.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

1/My first question concerns a point that is not addressed at all in the manuscript and which perhaps should have been included in the manuscript. It concerns image processing. I know that you have undertaken many efforts o to develop image processing software/macro.Why have you overlooked this fundamental point, both in the formatting of the image from the raw data, but also in the image processing (selection of areas of interest, integrations, statistical calculations, etc.)?

2/ My second question or comment is related to the washout time of the ablation cell, which is an extremely important factor to study in order to optimize image distortion and acquisition time. Without wanting to minimize the quality of the manuscript and the work done, which is excellent, I think it would have been useful to discuss this further, both in the manuscript and in the published articles. Why not systematically compare the ablation parameters studied (sample speed, beam diameter, repetition rate) with the wahsout time of the ablation cell, so as to make the operating conditions used more universal with other ablation systems?

3/ You insist on the fact that shortening the analytical time for acquiring an image by LA-ICPMS is of importance. Apart from the rinsing capacity of the cell and the speed of detection of ICPMS which will have to be improved in multi-isotopic mode (except for the ICP-TofMS that are fast but much less sensitive than the quad or HR ICPMS), what do you think is the limit in terms of the capacity of the ICP module to tolerate very high ablation rates, and how can it be evaluated (and eventually improved)?

4/The next question is more specifically dedicated to the choice of th quantum Dot (CdS). What is the main reason why CdS quantum Dot were used instead of AuNp? This question because, Cd and Au have more or less the same high ionization potential (9V and 9,2V respectively) allowing only about 50-60% ionization efficiency. However, Cd was monitored via 111Cd whose abundance sensitivity is only 12,8% in contrast to 197Au (100%).

5/ My last question concerns the MIP approach which looks very promising although I see some limitations. Referring for instance to paper 15, page 203, you claim that since the sensitivity is higher for concept B, limit of detection (calculated as 3 SD of the gas blank) is also better and this approach is the most convenient.
However, you show that the net Cd signal ratio from MIP/NIP (which somehow corresponds to the method signal to background ratio) is about 13,5 for concept A and 3,4 for concept B. Why not considering the signal to background ratio which might be more relevant thant the sensitivity alone?
You calculated the LOD based on the gas blank value… Wouldn’t it be more realistic to consider the NIP washed after CdS and/or sample deposition as a blank?

Finally, referring for instance to paper 15, page 204, figure 5 I wonder how you calculate the IgG concentration in a real sample: do you need to measure the signal first on the MIP and then on the NIP and make the difference?

Conclusion

There is no doubt that the manuscript presented is of a very high scientific standard. Dr. Tomáš Vaculovič is an experienced researcher who brings a lot to the scientific community through the originality of his work.
Consequently,the habilitation thesis entitled “Laser ablation as a powerful tool for determination of elemental distribution” by Tomáš Vaculovič **fulfils** requirements expected of a habilitation thesis in the field of Analytical Chemistry.

Date: Signature:

Pau, 19 August 2020