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Review Report

on the Ph.D. thesis submitted to the Discipline of Chemical Sciences Council at Faculty of Chemistry of Nicolaus Copernicus University in Toruń to attain the title of Ph.D. – Doctor of Philosophy in Chemical Sciences entitled "Novel approach for Low Molecular Weight Compounds analysis using nanomaterials-assisted Laser Desorption/Ionization Mass Spectrometry and Solid-Phase Microextraction"

Author: Radik Mametov, MSc (Candidate)
Supervisor: Dr. hab. Paweł Pomastowski, NCU Prof.
Co-supervisor: dr. hab. Aleksandra Radtke, NCU Prof.

The legal basis for this review is the Act of July 20, 2018, Higher Education Law and Science with amendments.

The research was carried out primarily at the Interdisciplinary Center for Modern Technologies of the Nicolaus Copernicus University. The doctoral dissertation includes four published scientific works. Candidate's publications P1 to P4 were published in Scientific Reports (two articles, impact factor – 4.6; publisher - Nature Portfolio, previously Nature Publishing Group), International Journal of Mass Spectrometry (IF = 1.9, Elsevier) and Materials (IF = 3.7, MDPI). The total impact factor of these four publications is almost 15.

In the initial part of the dissertation, the Candidate provided a very short introduction to the topic of the work, then presented quite narrowly formulated research problems. After the introduction, the aim of the work was presented and then the publications that were the basis of

the doctoral thesis were presented. The final part briefly describes the most important achievements and conclusions from the research.

The first research problem addressed in the work is the development of new fiber coatings of fibers for solid phase microextraction (SPME) with final application in gas chromatography (GC). The possibility of using metal-organic frameworks for modification of polypyrrole coating was studied. New coatings were tested with special emphasis on the extraction of low molecular weight compounds which was the core material of the P1 publication of this doctoral dissertation. Candidate presents the extraction performance of a new coating using known analytes and then tests its application for the extraction of volatile organic compounds (VOCs) emitted by *Enterococcus faecalis* bacteria. The reviewer highly values the results related to the production of new SPME coatings and their characterization, and has no objections to the tests of their effectiveness. However, the GC results presented in this work for VOCs emitted by *Enterococcus faecalis* are puzzling: Table 3 in the publication P1 presents ‘volatile organic compounds’ and yet contains nonvolatile compounds such as cathinone or alanine. Other questionable compounds are presented in Table S1, for example, ‘ethyne, fluoro’ (fluoroethyne?), 4-fluorohistamine, hexamethylcyclotrisiloxane, *p*-bromofluorobenzene - all are not of biological origin, additionally also norpseudoephedrine, *N*-dl-alanylglycine and pterin-6-carboxylic acid - are not volatile. Candidate should explain the origin of these compounds.

Work **P2** is focused on the synthesis and characterization of gold nanostars and their application for the detection of low molecular weight compounds with the use of commercial MALDI ToF-type laser mass spectrometers. The reviewer highly appreciates the presented results, which prove that the method is useful for LDI mass spectrometry. However, the Candidate has to clarify a few matters such as:

1. What value of the m/z error was acceptable and what was unacceptable in lipid tests? For example, palmitic acid was found as a potassium adduct at m/z 295.34 while the calculated value is 295.20339 which corresponds to an error of almost 500 ppm. With properly calibrated reflectron, instrument errors should be in the 1-5 ppm range.



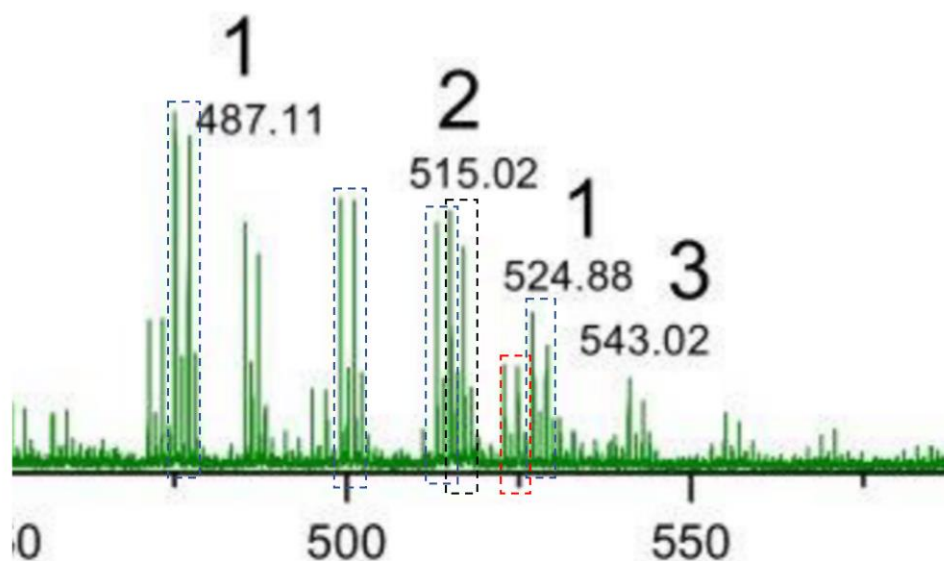
2. PI 18:0/20:3 has sodium adduct at 911.08 visible on the spectrum but 911.42 in the text. The isotopic pattern of this ion does not match the calculated one (based on the spectrum visible in publication). It is stated that $[M+H+2Na]^+$ ion was found but how it is possible with this structure? After the labile phosphate proton with sodium, the formula would be $[M-H+Na+Na]^+$.
3. What ion types or ion formulas were considered valid?
4. On page 8 of the publication, there is a statement that lyso-PE 16:1 lipid was found as an adduct with proton and two 'sodium atoms' with single positive charge at m/z 498.27. Please provide the structure of this ion.
5. Page 8 states that "lysophosphatidylethanolamine showed the adduct with *seven* atoms of sodium and five molecules of water at m/z 732.29" with quite low intensity. This is a very puzzling finding that lipids are capable of forming ions with so many sodium atoms (or rather sodium cations?). And this ion is having a single positive charge. Please provide the structure of this ion.
6. Similar case is with lyso-PE 16:0 with "two atoms of potassium and two molecules of water" Please provide examples of structures for these ions with calculated and experimental m/z values.

The purpose of the publication **P3** was to present research results on silver nanostructures for LDI MS of low molecular weight compounds. Silver coatings of the LDI MS targets were generated by the CVD method developed locally. The nanostructures were then characterized with X-ray diffraction, SEM microscopy, and of course laser mass spectrometry to confirm the applicability of the new method. Also, here, the reviewer believes that the results are interesting and valuable. However, there are some ambiguities that need clarification:

1. On page 68, Chapter 3.3, there are ion formulas for the detected lipids: ' $[M-2H]^-$ '. The reviewer is asking for an explanation why, after removal of two protons, there is only one negative charge? A similar situation exists for ' $[M-3H]^-$ '. Please provide examples of structures for these ions with calculated and experimental m/z values.



2. Page 70 contains, twice, formula $[M+Na+H]^+$, Please provide examples of structures for these ions with calculated and experimental m/z values.
3. Supplementary materials Table S1 also contains ion formulas such as: $[M-3H]^-$ for PE lipids, $[M-2H]^-$ for PE and TG lipids. Please provide examples of structures for these ions with calculated and experimental m/z values.
4. Supplementary materials Table S1 – cholesterol was assigned to negative radical ion at m/z 385.83. However, the calculated value is 386.35541. Please explain this difference.
5. Silver-107 cholesterol adduct has calculated m/z of 493.25941 and to this value 493.41 was assigned. That is a very large mass error of ~300 ppm and therefore this assignment is not valid for a calibrated spectrum. Other compounds should also be checked.
6. Supplementary materials Table S1 – amino acids were detected in negative mode as anion radicals while these are zwitterionic ions that are typically detected in mass spectrometry as classical, $[M-H]^-$ ions which is natural behavior for their ionic character. The same conclusion can be reached for the carboxylic acids. Can Candidate provide an explanation to this matter?
7. The serine anion radical should have m/z near 105.04, not 106.08.
9. Deuterated LysoPC-d5 17:0 with proton should be at m/z 515.38680, not 515.02. LysoPC 19:0 at 543.41810, not 543.02 (page 70). LysoPC 15:0 with sodium cation should be at m/z 520.26702, not 598.90. LysoPC 15:0 with potassium cation should be at 525.31138, not 524.90. Additionally, the spectrum provided for this region suggests that there are many classical silver adducts of characteristic isotopic pattern (100% signal/lower signal/93% signal/lower signal) visible in this area, and matching lipids to H^+ or Na^+ or K^+ adducts should be made carefully (dashed lines):



Publication **P4** describes the results of research on metabolic profiling of bacteria with polypyrrole-metal-organic framework SPME fibers and plasmonic nanostructured LDI MS substrates. In this work, extraction is followed by SPME-GC-MS analysis for characterization of bacterial VOCs. Also, NALDI MS of bacterial extracts was made for the characterization of nonvolatile compounds. The NALDI results were also compared with the MALDI results. The application of both methods is theoretically a very good idea that allows for much wider characterization of small compounds. The data obtained from the measurements were analyzed with principal component analysis (PCA), random forest analysis (RF), hierarchical cluster analysis (HCA), and canonical correlation analysis (CCA). This allowed the differentiation of bacteria species at a higher level than that of GCMS or NALDI MS alone.

The methodological or nomenclature deficiencies indicated in this review were presented due to the reviewer's obligation and serve to improve the Candidate's skills. The reviewed doctoral thesis is a valuable source of knowledge, and the evaluation of the entire dissertation is positive.

In summary, I can state that the reviewed doctoral dissertation meets the requirements of 20 the Act of July 2018, Higher Education Law and Science with later amendments. The experimental program conducted by the Candidate and the diversity of the research methods



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significantly expanded the Candidate's research competencies, enriching his research skills. Radik Mametov, the author of the thesis, proved to have the ability to conduct scientific research. The presented doctoral dissertation constitutes an original solution to a scientific problem and meets the requirements of the Act. Therefore, I am applying for admission to the public defense.

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