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Review Report on Ph.D. Thesis of Mr. Hussam Al Saoud

entitled:

“Biosilica as a New Packing Material for Chromatographic Separations”

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Mr. Hussam Al Saoud submitted the Ph.D. thesis dealing with the diatom frustules (containing biosilica) considered as a stationary phase for separation science, particularly for liquid chromatography, with the indication on the selected comprehensive applications in the area of bioanalytics and analytical chemistry. One of the crucial points of the Ph.D. dissertation is also to make applicable the novel biosilica-based technology as the promising new tool from the point of view of so-called “green chemistry”.

Generally, the novelty as well as scientific level of the Ph.D. thesis is well addressed, considering the importance of the research subject, technical requirements and perspective demands. The dissertation is well balanced in structure and correctly presented. It consists of ten main chapters. Summary in Polish, scientific activity of the Ph.D. student and appendixes (the papers and manuscripts submitted to publication) are presented among that. The thesis is written with the reasonable number of 108 pages altogether, and it is enriched by number of figures and tables shown mostly in the main bodies of the papers and manuscripts. The

theoretical principles as well as the research part were validated with 34 valuable, additional references collected in the dissertation.

The Ph.D. thesis in the first line is typically composed of an introductory part (Chapter I – Introduction, Chapter II – Aims of the study and Chapter III – Research problems), a part of experimentally processed data in the context of the commented results along with the discussion in the form of papers and manuscripts submitted to publication (Chapter IV) as well as final conclusions (Chapter V). The description in an introductory part primarily concerns basic information on main ideas fundamental to perform research (Chapter I). Further, just after the properly and shortly formulated aims of the study (Chapter II), the Author devotes quite a lot of attention to the presentation of principles of selected research problems encountered during the research (Chapter III). It is good idea and the focus is on the explanation of the diatom biosilica as a promising material in separation sciences as the first task. In a transparently short discussion, the methodology for the developing of a new stationary phase for liquid chromatography is next commented. The important description of HILIC separation mode to be applied for a new stationary phase is depicted, too. And finally, some basics of applications of biosilica as a potential packing material in the solid phase extraction is showed. Preparation procedure of biosilica and the technology of column packing are at last the valuable fragments complementary to the rest of the Ph.D. thesis description. In overall, in this part of the introductory, useful piece of information regarding the individual, unique properties of diatom biosilica taken from diatom frustules (exoskeletons) and their potential application pathways in analytical chemistry are presented. To summarize that introductory part of the Ph.D. thesis, it is worth to note that the Author has studied carefully a research subject with the critical view, and he used an appropriate number of bibliography sources of high quality, involving his own, too. Generally, this part of the thesis is well written, clearly and completely collected. It introduces the reader to continue further investigation into more methodological, paper-based chapters very well. It is an evident fact that Mr. Hussam Al Saoud deeply understood the theoretical knowledge and discussed problems.

In Chapter IV, the scientific exploration performed by the Ph.D. student is thoroughly discussed and shown in the form of corresponding manuscripts and papers. In the opinion of the reviewer, the most three important achievements of the dissertation could be considered as following.

1. The design of the new stationary phase was based on the diatom biosilica taken from diatom frustules, specifically modified with the octadecyldimethylchlorosilane to produce RP mode-suitable stationary phase with the final evaluation of the chromatography separation with selected six alkylbenzenes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, and *n*-pentylbenzene) plus the evaluation of chromatography goodness experienced just with anthracene.
2. The proposal of the other new stationary phase was based on the diatom biosilica taken from diatom frustules, without any specific chemical modification, but with well-preserved frustules without debris, to produce HILIC mode-suitable stationary phase with the final evaluation of the chromatography separation with selected five polar compounds (adenine, adenosine, guanosine, cytosine, and inosine).
3. The review papers (both, written in English and Polish) provided interesting fundamentals of the chemistry of diatom frustules and their potential usefulness in analytical chemistry.

The appropriate data analysis, interpretation and discussion provides new insights into the subject of new columns' synthesis and the possible assumptions to think about novel stationary phases in perspective. Based on the results obtained nowadays by Mr. Hussam Al Saoud, the Ph.D. dissertation made the next, substantial progress to support the further understanding of mechanisms regulating the observed physicochemical effects in the considered diatom biosilica frustiles-based stationary phases. Consistent results make the realistic perspectives for further, even deeper discussion useful from practical point of view. At the end of Ph.D. dissertation, the general conclusions of the performed research are correctly described. The thesis is clearly written and well documented. Although one can find some typing errors, the text is finally clearly constructed in elaboratively effective and consistent manner. The figures, equations, and tables are shown properly as well. The hypothesis and arguments are well formulated with meritorious conclusions based on valuable and actual literature, involving those ones with the Author's direct contribution. The conclusions confirm that the generated objectives of the work are successfully completed.

Below there are some remarks/comments which occurred to me and need to be explained in details or thoroughly discussed.

- 1) The diatom biosilica were taken from diatom frustules originally produced in the laboratory environment. The physicochemical properties of biosilica used in the considered research have been already characterized previously by Sprynskyy *et al.* and described in other papers. Firstly: is it the need to check any properties of the biosilica before the use them in the analytical chemistry to confirm their proper ability for a required separation science usefulness? Secondly: is any knowledge on any problems with the reproducibility of the biosilica properties produced by diatom frustules?
- 2) Modification of biosilica with the octadecyldimethylchlorosilane is the significant point in the research to produce RP mode-based stationary phase. It is provided that coverage density parameter of the Biosilica C18 has even higher value in comparison to the HALO C18. Is any assumption what Authors' procedure details influenced on that? On the other hand, the separation efficiency and symmetry of anthracene is claimed as worse one obtained with Biosilica C18 in comparison to the HALO C18. Is it easy to check and/or confirm that the mentioned end-capping in the commercially available product can be the solution for that problem also for biosilica? Was the production of the new biosilica-based stationary phase tested for the process reproducibility? What asymmetry factors were noted for six PAHs – it is written it is an excessive tailing but without detailed data provided. What anti-inflammatory drugs were the subject of interest in the corresponding research?
- 3) High values of asymmetry factors were noted also for some test compounds separated on the biosilica-based stationary phase in HILIC mode. Those values were noted for isocratic mode commented finally in the text as less successful ones than the adopted further the gradient mode shown in Fig. 2. What asymmetry factors can be found for five separated analytes chromatographed with the gradient mode and what ideas can be possibly proposed to improve asymmetry factors in the next steps?

To sum up, the dissertation thesis completed by Mr. Hussam Al Saoud represents high level scientific work. In my opinion, it is interesting topic for scientists working on analytical chemistry. All experiments are well arranged, measurements and analytical chemistry techniques as well as methods are correctly applied. They are generally well presented and flexible to follow. The explanations are suitable and focused on the relevant topics. It is noteworthy that the wide spectrum of work executed represents new research ideas.

I would like to emphasize that Mr. Hussam Al Saoud's dissertation is worth of attention due to the following aspects: scientific novelty, an extensive range of research, meritorious presentation and discussion of obtained results, and the perspective for the higher scientific activity confirmed by the submitted manuscripts to be published probably in commonly known and highly ranked scientific journals (*Journal of Separation Science, Journal of Chromatography A, Chromatographia*).

In my opinion, the reviewed thesis fulfills all requirements posed on Ph.D. theses aimed also for obtaining Ph.D. degree by Mr. Hussam Al Saoud. Hence, my opinion is positive, and I state that the Ph.D. dissertation is ready to be defended, in front of respective committee of the Nicolaus Copernicus University in Toruń.

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Kierownik