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Review of the PhD thesis entitled "**Electrochemically synthesized functional polymers in macromolecular architectures and diagnostics**", submitted by Nabila Yasmeen

The doctoral thesis submitted by Ms. Nabila Yasmeen presents the scientific data corresponding to circa 4 publications. In fact, 2 papers based on these data have been already published and 2 other papers have been submitted to journals. The published papers appeared in very good scientific journals: *Analytica Chimica Acta* and *ACS Applied Polymer Materials*. Ms. Nabila Yasmeen is the first author in all mentioned above papers. The submitted thesis is of medium size (159 pages) and contains 15 schemes, 60 figures, 342 references and some other graphs.

The material presented in the thesis is in my opinion actual, important and with a good potential for successful applications. Ms. Nabila Yasmeen has synthesized several advanced polymers and gels, also in the micro and nanosize, and used a variety of methods and techniques to characterize the structure and the activity of the obtained materials. The methods used included dynamic light scattering, scanning electron microscopy, scanning transmission electron microscopy, atomic force microscopy, confocal microscopy, voltammetry and chronoamperometry, diagnostic computational calculations and simulations that helped in selection of appropriate monomers, and finally handling of bacteria. No doubt, one long and 2 short scientific visits of the PhD student in good foreign laboratories helped in learning so many tools.

Complete lists of abbreviations and symbols are added to the thesis and this helps in the reading process.

The thesis starts with an introduction. The introduction is rather voluminous, since the author works with several materials and investigates several problems. In the beginning Ms. Nabila

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Yasmeen indicates that the needed performance of the functional polymers can be reached when appropriate actions at the synthesis step are introduced. Just because of their sensitivity to various chemical groups and environment conditions they are extensively used for therapeutic and sensing applications.

Then Ms. Nabila Yasmeen notes that functional polymers are widely used in molecular imprinting, because relatively easy removal of template molecules from the surrounding polimer generates molecular cavities which become the sites capable of selective binding the analyte molecules.
The functionalized polymers are becoming also frequently used as matrices and supports. In consequence, the materials made of functional polymers become the foundation stone of innovative technology. Ms. Nabila Yasmeen focussed on two major issues in her work: supramolecular, responsive and functionalized polymers serving as the imprinting matrices, and synthetic base membranes for use as a microenvironment for three-dimensional formation of cell culture.

The title of the next chapter is "Experimental section". This chapter is also rather long. The thesis author presented here all used techniques, described the instruments and the procedures, and also addressed the bacterial strains and their growth conditions.

The main chapter in the thesis is Chapter 3. It is divided into four subchapters and contains all obtained results and the corresponding discussion.
In the first subchapter the author described the creation of an electrochemical MIP chemosensor for selective determination of E. coli E2152 strain. The sensor was positively tested. The key step in the construction was successful imprinting of E. coli E2152 strain in a polymer using polymerization with electrochemical initiation. 2-aminophenyl boronic acid was selected as the functionalized monomer and aniline served as the crosslinking monomer. It was possible to conclude, after the analysis of the obtained SEM micrographs, that just in a single step the bacteria template could be completely entrapped in the molecularly imprinted polymer matrix. Slightly more complicated was the removal of the bacteria from the MIP matrix. A three-step procedure had to be used to effectively vacate molecular cavities in the molecularly imprinted polymer. The removal of the template could be examined using the SEM technique. From analytical point of view the performance of the sensor was satisfactory.

The linear dynamic concentration range for the E. coli extended from 2.9×10^4 to 3.1×10^7 cells mL^{-1} with the correlation coefficient (R^2) of 0.984.

The work described in subchapter 3.2 is focused on the determination of gamma-aminobutyric acid. This detection is important for clinical analysis. The aim of Ms. Nabila Yasmeen was to develop a simple, inexpensive electrochemical chemosensor. The preparation procedure consisted of electrochemically initiated polymerization with involved molecular imprinting. Potentiodynamic conditions for the electropolymerization were selected. To choose appropriate synthetic receptors for MIP preparation some computational modeling was done. At first, regular Pt disk electrodes were used in the process. Later it appeared that Pt disks can be substituted with disposable commercial interdigitated electrodes, which should be useful in point of care applications. The developed method was also examined in real serum samples. The results were promising. In my opinion good sensitivity, linearity, and selectivity of the method was the result of perfect employment of the electrochemical impedance spectroscopy technique.

Subchapter 3.3 is focused on preparation of innovative eco- and bio-friendly gel structures with the use of simple green synthesis procedure. The aim was to develop a material suitable for microbiological applications including drug delivery and tissue engineering. Ms. Nabila Yasmeen used the electrochemistry techniques and successfully developed an alternative way to prepare a variety of polyacrylamide microgels and core-shell particles. The material of the cores was inorganic; either silica or magnetic nanoparticles were employed. The surfaces of those inorganic cores were covered with shells that consisted of the NIPAM-MA-BIS copolymers of various stoichiometry. Thickness of the shells was in the range of nanometres. In particular, the potential of electroreduction of the initiator and the molar fraction of the crosslinker were optimized to get the most useful morphology of the nanoparticles. In fact, not surprisingly, concentration of BIS was the major factor influencing the final morphology of the gel particles. Within a wide characterization of nanoparticles, thermal stability was also examined.

The use of the nanoparticles described in subchapter 3.3 for 3D cell-culture formation is reported in detail in subchapter 3.4. Ms. Nabila Yasmeen noticed that by changing amount of the crosslinker in the gel, it is possible to modify its solubility, softness and finally diffusion of ions within the net. So, the gel NPs could be biocompatible and could mimic the biological

environment for tissue culture. It appeared that the use of the NIPAM-MA-BIS gel NPs as the base/support resulted in significantly extended 3D structure growth compared to the classical control. If fact, for both synthesized gel systems, i.e. silica core-NIPAM-MA-BIS and magnetic core-NIPAM-MA-BIS core-shell gel nanoparticles the provided support was much more beneficial than the conventional glass plates. The author concludes that the core-shell gels are a promising tool for studying more complex 3D cell cultures.

The last chapter before the references list is entitled "Conclusions and future outlook". It is in fact an extended summary decorated with very optimistic perspectives regarding the obtained new micro- and nanomaterials. A long list of possible applications is given: drug and gene carriers, tissue engineering, sensors and biosensors, bioseparation of active molecules and microfluidics. I support that optimism.

There are several issues I would like to discuss with the thesis author during the defence. They are as follows:

- Figure 2-6 presents an n-th voltammogram from multiple scan experiment, while it should be the first-scan curve.
- Figure 2-7 instead of DPV waveform and peak illustrates these features for SWV.
- Experimental DP voltammograms should be characterized in figure legends with pulse time/width and period between pulses. Peak height is also important.
- Eqn. 2.2 should precede eqn. 2.1.
- It should be explained in the thesis why instead of a regular reference electrode an Ag quasi reference electrode was used in some electrochemical experiments.
- Figure 2-12 would be more informative if successive scans were added to it.
- The graphs on pages 72, 88, 102 and 118 have no label.
- (3.3.1) Why the synthesis of polyNIPAM was done under vigorous magnetic stirring and in the presence of higher, than usual, persulfate concentration?
- Correlation coefficients are presented for the gels in Figures 3.3-7 and 3.3-8; however, they are not discussed in the text and no corresponding data are given. Anyway, the correlation coefficient obtained for the gel microparticles at pH 6 (Figure 3.4-8) is more complex.

In summary, I would like to say that the submitted PhD thesis contains apparent elements of scientific novelty. The presented experiments and the derived conclusions should be useful for

the people working in the functionalized polimers/gels area. I have no doubts that the thesis presented for the evaluation meets the requirements for doctoral dissertations given in article 187 of the Act of July 20th, 2018, on law on higher education and science (Journal of Laws/Dz. U. (2018), item 1668 as amended). Therefore, I request that Ms. Nabila Yasmeen be admitted to the next stages of the doctoral procedure.

Warsaw, May 27th, 2023.

Prof. Zbigniew Stojek