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Review of the doctoral dissertation by Nabila Yasmeen

entitled: 'Electrochemically synthesized functional polymers in macromolecular architectures and diagnostics' prepared within the International Doctoral Studies in Chemistry at the Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland, under supervisions of:

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The development of analytical methods based on physicochemical phenomena has led to reduction of detection limits and increase of precision of the measurements of analyzed samples. The use of specific detectors and implementation of such detectors into analytical protocols resulted in the repeatable determination of analytes at very low concentration levels. However, biological matrices are still challenging, hampering further application of detectors because of high complexity of matrices, such as body fluids (urine, saliva or plasma). Apart from the sample collection, all of them required individual protocols to clean-up sample prior to analyte detection. Those additional steps of analytical protocols are error-prone, laborious and time-consuming, affecting the analytical performance of the method. For such purpose, investigations of new and advanced materials for fabrication of specific detectors are highly justified. It could be supposed that such detectors result in further progress of the analysis of clinically important biomolecules and/or biomarkers of pathological states, providing additional value to human's life as well as to health-care systems. Here, functional polymers could be considered as attractive alternative to already existing materials because they often provide properties such as conductivity, photosensitivity or pharmacological activity. Moreover, they could be designed in a way to respond to external physical or chemical factors or biological stimuli. Here, molecularly imprinted polymers (MIPs) could be considered as a group of advanced functional polymers that provide satisfactory selectivity, high thermal and chemical stability as well as that are characterized by satisfactory reusability and clean-up capabilities.

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Those properties determine the application of MIPs, mostly in the separation science and detection methods.

The doctoral dissertation by Nabila Yasmeen presents an investigations aimed to prepare the selective polymeric materials, employing, mostly, a molecularly imprinted technology. The studies by Nabila Yasmeen were carried out in a group of Prof. Włodzimierz Kutner, comprising a part of long-lasting investigations related to synthesis of molecularly imprinted biosensors based on electropolymerization of heterocyclic systems.

The doctoral dissertation by Nabila Yasmeen has a typical layout. It consist of 159 pages with the following chapters: introduction together with the thesis objectives, experimental section, results and discussion, conclusion and future outlook and references.

In the introduction Author describes the fundamental knowledge related to MIPs, the synthetic strategies, including electrochemical MIPs synthesizing, the molecular imprinting of low molecular weight compounds as well as macromolecules prior to detail description and characterization of polymeric gels. The experimental part describes, in detail, data necessary to laboratory studies, focusing mainly on the instrumental techniques and protocols that were applied during the work. The results section critically discusses four aspects of studies, viz. MIPs as a receptor mimic for capacitive impedimetric selective recognizing *Escherichia coli* K-12, electropolymerized MIP for chemo-sensing of gamma-aminobutyric acid as an autism biomarker, electrochemically initiated synthesis of polyacrylamide gel microparticles and core-shell nanoparticles, and electrochemically synthesized polyacrylamide gel and core-shell nanoparticles for 3D cell culture formation.

The thesis objectives were defined as synthesis of functional polymers and nano- and microgels oriented towards clinical analyses under electrochemical conditions. The scopes of the work based on the identification of current limits of molecular receptor biomimetics, including functional complexity, fragile structures, harsh conditions for template removal, and low selectivity. Aspects of 'green-chemistry principles' were also taken into account. The research plan was presented.

The first section of results and discussion is devoted to MIP as a synthetic receptor mimic for capacitive impedimetric selective recognizing of *Escherichia coli* K-12. Here, Author describes the synthesis of polymer composed from 2-aminophenylboronic acid and aniline, using a bacteria cell as a template. The results contain electrochemical characterization of the synthetic process, the investigations of the interactions that could govern the recognition of specific regions on the bacterial cell wall, detail optimization of the bacteria cell template removal, physico-chemical characterization of material, using scanning electron microscopy, X-ray

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electron dispersive spectroscopy, atomic force microscopy and electrochemical measurements. The selectivity studies were carried out towards three interferences, viz. *Shewanella oneidensis* MR-1, *Escherichia coli* E2146 and *Escherichia coli* E2498.

It was found that the electrostatic attractions are responsible for the binding in the cavities and the bacteria cell template removal proceed only when the entire cleaning protocol is employed. In summary, it was stated that the excess of the cross-linker maintained the geometry of cavities after removal of bacteria cell template.

Here two interesting questions could arise. First question is related to findings that mild conditions during the removal of bacteria cell template were ineffective. Why? Second question is related to the participation of amine groups from the cross-linker structure in the interactions with macromolecules that are present on the bacteria cell wall. I appreciate Author's answer to those questions during a public defense of the doctoral dissertation.

The second section of results and discussion is devoted to electropolymerization of MIP for chemo-sensing of gamma-aminobutyric acid autism biomarker. Here, Author performed the theoretical analyses of interactions of five functional monomers in the prepolymerization complex with template molecule of gamma-aminobutyric acid prior to the synthesis of preselected monomer in the presence of cross-linker. The electropolymerization of derivatives of 2,2'-bithiopehene systems was carried out together with characterization of fabricated material, template removal monitoring, and selectivity studies, using components of model artificial serum, viz. 5-aminopentanoic acid, 2-aminobutanoic acid, N-acetylaspartate and glucose.

It was found that the resulted polymer was composed of granules, providing high extension of the film surface as well as sufficient roughness. However, due to the polymer chain's stacking, the template removal process resulted in elongated fibril-like structure. Satisfactory selectivity were confirmed with imprinting factors exceeding two.

Here, from the reviewer responsibility, I shall recommend that the guidelines for nomenclature of organic compounds should be applied, for instance, FM1: 4-bis(2,2'-bithien-5-yl)methylbenzene-1,2-diol or FM4: 4-bis(2,2'-bithien-5-yl)methylphenoxyethanamide.

At this point, I appreciate Author's comment to the following problem. Autism spectrum disorder is a neurological disfunction that affects people's interactions with others, such as communication, learning, and behavior. It is a complex disorder that still requires comprehensive investigations to be fully known. However, the current knowledge identify several different biomarkers that could be related to autism spectrum disorder. Thus, from the biomedical point of view, it could be interesting to determine not only one biomarker selectively

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but provides a tool that allows us to detect various biomarkers and, most interestingly, allows us to provide data related to mutual ratio of those biomarkers since the mutual fluctuations between selected biomarkers could reveal pathological states more precisely. Thus, in the context of above, please discuss the usefulness of the designed detector.

The third section of results and discussion is devoted to electrochemically initiated synthesis of polyacrylamide gel microparticles and core-shell nanoparticles. Here, Author presents a simple procedure for gel synthesis, using methacrylic acid, N,N-methylene-bis-acrylamide and N-isopropylacrylamide, discusses a potential mechanism of the reaction and provide data from scanning electron and transmission electron microscopies and nitrogen sorption data (Brunauer-Emmett-Teller and Barrett-Joyner-Halenda models) to reveal morphology, and data from infra-red, solid state nuclear magnetic resonance, X-ray energy dispersive spectroscopies and thermogravimentry to confirm the structure of obtain materials.

It was found that both, thin film and silica core-shell materials were obtain and comprehensively analyzed confirming their composition, structure and morphology.

Here, from the reviewer responsibility, I shall indicate that the abbreviation MA was used to code methacrylic acid but MA is not decoded in the abbreviation list where MAA exists. Moreover, the structure of compound coded as MA in Fig. 3.3.1 refers to acrylic acid but not to methacrylic acid.

Here, the tentative mechanism proposed by Author reveals formation of primary radicals as reactive individuum in the polymerization process. I appreciate Author's comment to the stability of radicals with respect to basic knowledge that secondary or tertiary radicals are more stable individuum.

Last, four section is devoted to electrochemically synthesized polyacrylamide gel and core-shell nanoparticles for 3D cell culture formation, describing further synthetic optimization to improve the morphology and size of these particles as well as to improve the gel particles' architecture, in order to obtain a 3D culture basement membrane-like extracellular matrix. Finally, the biocompatibility tests were performed, using in vitro cytotoxicity assays on two different cell lines, viz. MDA-MB-231 (triplenegative breast cancer) and HeLa (cervical cancer) using the MTT proliferation/(metabolic activity) assay.

It was found that obtained gel nanoparticles dispersed in acidic and neutral solutions are nontoxic and highly biocompatible for both cell lines in a broad concentration range.

In the conclusions, it was stated that an *Escherichia coli* E2152 strain was successfully imprinted in a polymer matrix, using electrochemically initiated polymerization of 2-aminophenylboronic acid that acted as the functional monomer and aniline – the crosslinking

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monomer. The interferences study indicated that the cell surface compositions mainly governed their binding behavior, and the inexpensive analytical method, exploiting different cell surface properties can be an excellent substitute for conventional biochemical approaches for effortless and quick pathogen identification in real-time. The electrochemical polymerization under potentiodynamic conditions was proposed to prepare a chemo-sensor for gamma-aminobutyric acid using molecular imprinting technology. The studies using artificial serum samples as a model complex medium, confirmed the possibility of the chemo-sensor application for real samples analyses. Finally, a simple green synthesis procedure was developed to prepare more advanced eco-friendly gel structures for microbiological applications. Electrochemistry was successfully exploited to pursue an alternative way to prepare different polyacrylamide microgels and core-shell particles.

Here, in my opinion, the novelty of presented studies (although undoubted) shall be clearly emphasized in the summary section.

The reference section contains 342 citations. The references, mostly originated from last two decades, were selected properly and comprehensively explain the background of studies described in the reviewed doctoral dissertation. Additionally, the lists of abbreviations and symbols are provided. Moreover, the graphical layout, multiple figures/schemes and tables make the dissertation easy to read.

The results presented in reviewed doctoral dissertation were published in the international journals, and were presented actively during the scientific conferences.

The doctoral dissertation by Nabila Yasmeen is written in English. Since English is not my native language, I do not comment style and grammar that was used by Author in dissertation. However, it has to be underlined that the highly specific scientific English language was used by Author properly.

Finally, I have to emphasized that the methodological background, laboratory practice and theory allowed Author to proceed smoothly through doctoral studies. The methods of synthesis and analysis of materials were selected properly, the results were discussed correctly, and the conclusions were formulated accurately. It should be also highlighted that the doctoral dissertation within its scopes presents innovative methodological solutions as well as utility with high probability to transfer of these studies for routine methods in future.

The questions and comments that I have expressed above, could be a part of the discussion and therefore an answer and an explanation will be appreciated during the public defense. At the same time I conclude that my comments and questions do not affect the level content and high scientific value of the dissertation.

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To sum up, the reviewed doctoral dissertation is an original work and provides a valuable data on the possibility of using functional polymers and biosensors obtained by the molecularly imprinted technology to the detection of important biomolecules. It is a creative contribution of Nabila Yasmeen to the development of polymer synthesis methods as well as chemical analysis in the field of construction and use of detection tools for clinical and biomedical applications.

Therefore, I believe that, in light of the applicable articles and regulations (Dz. U. 2018, poz. 1668), and, in particular, articles and regulations related to the academic degrees and scientific titles (Dz. U. 2003, poz. 595 z poźn. zm.), the presented doctoral dissertation by Nabila Yasmeen fulfills all articles and regulations requirements for doctoral dissertations. Thus, hereby I recommend to the Scientific Board of the Institute of Physical Chemistry, Polish Academy of Sciences to allow Nabila Yaseem to proceed further steps of the doctoral process.

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