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Review of the doctoral dissertation by Nabila Yasmeen

"Electrochemically synthesized functional polymers in macromolecular architectures and diagnostics"

The Molecular Imprinting Polymer (MIT) technique is considered a versatile and promising way to create materials with molecular recognition capabilities comparable to those found in nature. These systems can be dedicated to recognizing both molecules of biological origin (amino acids, fatty acids, hormones, DNA, RNA, vitamins, neurotransmitters) and chemical ones. The spectrum of application of these systems is very wide, ranging from separation and purification processes designing biochemical and chemical sensors, matrices for catalytic processes, and ending with drug delivery systems. MIT is synthesized by forming a complex between a matrix and a functional monomer. A three-dimensional polymer network is created in the presence of a significant excess of cross-linking agents. After the polymerization process, the matrix is removed from the polymer, leaving specific recognition sites complementary in shape, size, and chemical functionality to the matrix molecule. The resulting polymer recognizes and selectively binds only the matrix molecules.

The presented doctoral dissertation of M.Sc. Nabila Yasmeen entitled "Electrochemically synthesized functional polymers in macromolecular architectures and diagnostics" was performed at the Institute of Physical Chemistry Polish Academy of Sciences (IPC PAS). The promotors of the thesis were prof. Wlodzimierz Kutner, Ph.D., D.Sc. (IPC PAS), dr. hab. Piyush Sindhu Sharma, Ph.D. (IPC PAS) and dr. Mathieu Etienne, Ph.D. (CNRS, LCPME, Nancy, France). It should be emphasized that prof. Wlodzimierz Kutner and his team made a

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significant contribution to developing MIP systems. Their achievements are recognizable in the international arena. The research presented in the thesis follows this trend.

It should be noted that the subject of the doctoral dissertation is very topical and utilitarian. Designing new synthetic materials that can mimic the recognition processes occurring in nature is a significant and dynamically developing area of the potential application of molecular imprinting in recent years. The author's main goal was the synthesis of functional polymers and nano- or microgels in electrochemical conditions intended for use in clinical diagnostics. The research strategy included the electrochemical synthesis of MIPs dedicated to determining the autism biomarker of gamma-aminobutyric acid (GABA) (inactive electro neurotransmitter) and Escherichia coli bacteria. In addition, it was planned to synthesize polyacrylamide nanoparticles and gel polyacrylamide shells on selected inorganic cores and verify these nanosystems in terms of cell cultures.

The presented doctoral dissertation is written in English and is experimental. It covers a total of 159 pages and consists of 5 main chapters, including an introduction to the current state of knowledge on the strategy for obtaining and using MIT systems, a description of research methods, a presentation of own research results, and includes discussions, summary, summary, scientific achievements, and overview literature (342 items). The dissertation is richly illustrated with diagrams, drawings, and tables, making it easier for the reader to understand the adopted research concept and analyze the obtained results. From the editing point of view, the work is very well prepared.

The experimental part presents the analytical techniques used to characterize the synthesized MIP layers and nanoparticles and several measurement procedures. The description includes techniques such as: dynamic light scattering (DLS), scanning and transmission electron microscopy (SEM, STEM), atomic force microscopy (AFM), confocal microscopy (CM), electrochemical measurements (cyclic voltammetry (CV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS)), computational calculation. Such a variety of research techniques allowed the author to analyze the correlations between the properties of the obtained MIP-type polymer layers and their sensitivity, selectivity, and limit of detection in a multidimensional way.

Own works presented in chapter 3 focus on four main directions of research. They concern: the development of the MIP chemosensor to determine the Escherichia coli bacterium, the development of the MIP chemosensor for the GABA determination, and the synthesis of biocompatible polyacrylamide gel microparticles or core-shell nanoparticles.

The chemosensor MIP for the selective determination of Escherichia coli E2152 was developed based on the functional monomer of 2-aminophenyl boronic acid (2-APBA) and aniline (ANI) as a cross-linker. The resulting conductive MIP matrix showed effective immobilization of E2152 bacteria through interactions of boronic acid groups with cis-diol groups in the microbial membrane. The challenge was to develop a 3-step procedure for efficiently extracting strain E2152 from MIP to obtain an active template. The appropriate thickness of the MIP layer for the incorporation of bacteria was obtained after 12 electropolymerization cycles. The performance of the MIP layer imprinted with the E2152 strain was monitored using the capacitive impedance (CI) method. The MIP layer very effectively recognizes the analyte E2152. The indicator of E. coli binding to the MIP layer is the layer capacity and phase angle change. The dependence of the layer capacity on the E. coli concentration is linear in the range of 2.9×10^{-4} to 3.1×10^{-7} cells/mL. The system's selectivity was verified by tests for selected bacterial strains, which showed considerable differences in the system's response. A significant layer capacity was obtained for the EK2146, insignificant for SWMR-1, and no visible signal for EK2498. Upon removing the bacterial matrix, it was assumed that the MIP layer would recognize the target bacteria based on their shape, size, and the interaction between positively charged E. coli surface groups and negatively charged boronic acid-derived groups.

Functionalized bis(bitiophene) monomers were used to develop MIP targeting GABA recognition. The stability of the GABA complex with five functional monomers was assessed based on the Gibbs free energy determined using the density functional theory (DFT) with the B3LYP approximation. The most stable form was the complex formed with FM4 monomer (*p*-bis(2,2'-bithien-5-yl)methyl phenol 2-hydroxy acetamide ether) in the ratio 1: 2 (GABA to FM4). MIPs were immobilized on the surface of a platinum disc electrode and gold ring array (IDE). The chemosensor performance was verified by the use of three electrochemical methods (differential pulse voltammetry (DPV), capacitive impedance (CI) and

electrochemical impedance spectroscopy (EIS)). The sensitivity, selectivity, and limit of detection of the MIP layers were determined. The morphology of the deposited MIP layers on the IDE surface was compared with the layers of non-functionalized NIP layers using the AFM technique. The NIP layers show the presence of much larger surface aggregates concerning the MIP layers. The XPS method was used to control the GABA template. The spectra were analyzed by the component lines occurring in the N1s band. Lines from the protonated form of the amine group confirmed the presence of the GABA molecule in the layer. The prospect of the chemosensor's usefulness in clinical diagnostics was tested on artificial serum samples. In this respect, the most promising results were obtained with the EIS method.

Polyacrylamide gel microparticles and core-shell nanoparticles were obtained by electrochemical gel initiation. In this case, the electrochemical decomposition of ammonium persulfate has initiated the copolymerization of N-isopropylacrylamide, methacrylic acid and N,N'-methylenebisacrylamide monomers. Molecules morphologies with different NIPAM, MA, and BIS ratios were imaged using SEM and TEM microscopy. System properties were monitored by NMR spectroscopy, TGA, BET, and DLS measurements. The cytotoxicity of the polyacrylamide gel particles was performed with selected cell lines, MDA-MB-231 and HeLa. IC50 values were determined using MTT assays at pH 4.0, 7.0, and 9.0. SiO_2 nanoparticles and magnetic iron oxide nanoparticles are applied as the cores for core-shell systems. The formation of a gel coating on the surface of nanoparticles and its stability were confirmed using EDX and FTIR spectroscopy as well as TGA and DTGA. In the case of SiO_2 particles, the NIPM-BIS layer was more stable than MA-BIS and the NIPAS-MA-BIS layer on the surface of the magnetic particles. Confocal microscopy measurements showed that core-shell nanoparticles performed excellently as a substrate for complex three-dimensional tissue structures compared to a conventionally used substrate.

The doctoral thesis ends with a summary of the obtained measurement results and the prospect of applying the designed MIP systems. The research methodology developed by the doctoral student has great cognitive and application value. However, I would like the author to comment on several aspects that, in my opinion, have a significant impact on the discussed issue and the direction of further research:

1. On page 49, the author states that the DLS method can be used to characterize "solid particles". However, based on the author's work, it can be indeed indicated that it is also successfully used to measure "soft particles".

2. Chapter 2.4.7 on theoretical methods lacks a description of the DFT method, which the author successfully applied to optimize the GABA-based system. However, it should be emphasized that the use of computational techniques to select optimal structures is becoming a significant trend that is gaining more and more approval and is not only visual support of experimental methods.

3. The functionalization of the polymer layer was analyzed by verifying the presence of boron-derived lines in the MIP and NIP layers in the XPS spectra (chapter 3.1.6). Since the photoelectrons passing through the sample are scattered on the binding electrons and thus lose some energy, the XPS measurements only consider the sample's outermost layers. In the case of organic compounds, it is possible to analyze with XPS down to a depth of 3-10 nm. Assuming, based on the SEM analysis for bacteria E2146, that the thickness of these objects reaches 200 nm, thus preventing the observation of deeper layers. Indirectly, XPS measurements confirm the anchorage of bacteria in the matrix.

4. Based on the morphology of bacteria in the polymer layers obtained using SEM and AFM microscopy (Figure 3.1-2), we are dealing with elongated-shaped objects differing in length can be seen. It would be interesting to estimate the size distribution of such objects in the context of their immobilization in the MIP matrix. Moreover, from a practical point of view, it seems essential to identify whether the observed changes in the effectiveness of the immobilization of various bacterial strains (E2152, EK2498, SWMR-1, EK2146) mainly come from the variable composition of functional groups on the surface of the bacteria or the shape effect. As the author emphasizes, in the case of the E2498 strain, the system has a natural tendency to aggregate and consequently does not fit the template.

5. The dependence of the layer capacity on the E. coli concentration is linear in the range of 2.9×10^{-4} to 3.1×10^{-7} cells/mL; above this concentration, it reached plateaus. Therefore, it would be interesting to estimate the E. coli surface coverage in MIP, considering the size obtained from the AFM measurements?

6. At what angle were the XPS spectra recorded (Figure 3.2-4) for the MIP dedicated to GABA? Only the nitrogen spectrum was used for the analysis. However, using other spectral bands, it would be possible to obtain additional information about the polymer layer and assess the effect of the adsorption surface.

7. The topography of the MIP and NIP layers in Figures 3.2-8 is not readable. This is because the height of individual AFM images is not visible on them, and it is an essential parameter of the obtained layers. Presenting the topography on a 3D scale would improve the visualization of the spatial topography of both layers.

8. Sections 3.3.5 and 3.4.4 present the particle size distributions obtained using the DLS method. The analysis is presented depending on the intensity of light scattering. The question arises whether volume analysis should not be used for samples showing an increased PDI value.

9. Suggests in future research the use of the QCM-D method. This method will determine the viscoelastic properties of gel systems and the degree of their hydration, which is an essential parameter in biological applications.

The work is generally written in an understandable way to the reader, but there are a few mistakes in the text. The above comments do not change my positive opinion of the reviewed doctoral dissertation. The author showed that she could formulate research goals and plan experiments to achieve them. The author also perfectly uses modern scientific literature in the field of the presented topics. The choice of a wide range of experimental methods proves that Nabila Yaseen, M.Sc., is a mature scientist deserving a Ph.D. in Chemical Sciences.

The results obtained as part of the Ph.D. thesis of Nabila Yasmeen include four publications. Two publications have been published in renowned international scientific journals:

Electrochimica Acta (IF = 6.901), and Analytica Chimica Acta (IF = 6.558). The subsequent two publications are under evaluation. It is worth emphasizing that in all publications, M.Sc. Nabila Yasmeen is the main author. The author has participated in several conferences and research internships.

Summing up, the doctoral dissertation presented for review presents a significant value in terms of cognitive and application and thus has elements of novelty in the field of chemical sciences. Therefore, I conclude that the doctoral dissertation M.Sc. Nabila Yasmeen entitled "Electrochemically synthesized functional polymers in macromolecular architectures and diagnostics" meets all the requirements for doctoral theses specified in Art. 187 of the Act of July 20, 2018, on academic degrees and academic titles as well as on degrees and titles in art (Dz. U. z 2018r., poz. 1668 ze zm.). Therefore, I am applying to the Scientific Council of the Institute of Physical Chemistry PAS to accept the dissertation and admit M.Sc. Nabila Yasmeen to further stages of the doctoral dissertation.

The undertaken research problem required Nabila Yasmeen to develop a methodology for synthesizing new polymer systems using advanced electrochemical and analytical methods. Appropriately selected measurement techniques allowed the author to perform a multidimensional analysis of the correlation between the properties of the obtained MIP-type polymer layers and their sensitivity, selectivity, and detection limit.

The innovative approach to the use of MIP systems for the selective determination of biomarkers based on gamma-aminobutyric acid (GABA) and Escherichia coli bacteria and their high application potential should be appreciated. Due to the above, the presented Ph.D. thesis of Nabila Yasmeen deserves an award.

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