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Referee's Report on the Ph.D. Thesis
titled "***Carbazole and acrylate molecularly imprinted polymers and their application as recognition units in electrochemical chemosensors for selective determination of chosen drug substances***"
submitted by Jyoti, M.Sc.

The Thesis submitted by Jyoti, M.Sc. was prepared under supervision of prof. Włodzimierz Kutner and dr Krzysztof Noworyta. Its aim was to develop new chemosensors which could monitor concentration of some pharmaceutic substances in human plasma. In this kind of sensors so called "molecularly imprinted polymers" (MIPs) are applied, whose presence allows for selective recognition of analyzed species. Development and fabrication of such devices is a very demanding task since it embraces elements of theoretical, organic, physical and analytical chemistry.

The dissertation consists of more than 200 pages, including a list of 369 references. It is divided into seven main chapters: i) *Introduction* which in addition to a literature review contains the description of the background of undertaken research as well as the Author's motivations clearly stated; ii) very extensive *Experimental Section* describing chemicals, materials and experimental techniques; iii) four chapters presenting principal results and their critical discussion. The thesis is completed by a chapter titled *Research summary and future perspectives*.

In the first chapter the Author described state of the art in the domain of molecularly imprinted polymers, critically reviewing the literature, with the main focus being put on their application in chemosensors. Then, she concentrated on two specific groups of MIPs, which she had used for the fabrication of sensors. One of them was obtained by polymerization of

acrylic or methacrylic acids or their derivatives. The second group embraced conducting polymers (CP), which were electrochemically deposited on the surface of appropriate electrodes. The last part of the literature review was focused on analytical methods developed to date for the determination of two drugs in human plasma, namely Cilostazol (CIL) and Duloxetine (DUL).

Each of four chapters, devoted to the experimental part of the thesis, was closely connected to four papers co-authored by Jyoti (two of them already published and two submitted for publication).

In Chapter 3 the Author presented extensive study on two carbazole derivatives [9-(4-naphthalen-2-yl)-9*H*-carbazole (CNZ1) and 3,6-bis(thiophen-2-yl)-9-(4-naphthalen-2-yl)-9*H*-carbazole (FM3)] which were used as monomers and electropolymerized through anodic oxidation. The substitution of terminal carbazole 3,6-positions with thiophene rings in FM3 significantly changed its electrochemical properties lowering its oxidation/polymerization potential by *ca.* 0.4 V as compared to CNZ1. This lowering was predicted by quantum-mechanical calculations, carried out in support for experimental data. Electrochemical studies gave very interesting information, important for the new compounds application in MIP sensors. The presented spectroscopic and spectroelectrochemical results require, however, some comments. First, in the spectrum of CNZ1 (Figure 3-1) no spectral response can be noticed in the spectral range between 250 nm and about 310 nm. Is it connected with too high concentration of the monomer? Moreover, the statement that the spectrum of CNZ1 is red-shifted as compared to that of FM3 is disputable. Similar to the case of CNZ1, the least energetic band in the spectrum of FM3 presents a vibrational structure, although less pronounced. If the FM3 spectrum were presented as its second derivative ($d^2Abs/d\lambda^2$), the maximum of the *0-0* would be more clearly visible. It could follow from this analysis, that the *0-0* transitions were of similar energy for both monomers.

The products of CNZ1 and FM3 electropolymerizations were studied by cyclic voltammetry and UV-vis-NIR and EPR spectroelectrochemistry. Unfortunately, the UV-vis spectra of neutral polymers, which could provide information on the conjugation length in the studied macromolecular compounds were not shown in the thesis. They cannot be found in Figures 3-7 c and d, which only present spectral evolution (absorbance change) induced by the applied potentials. The discussion of the EPR results concerning the line width and g-factor is correct, however, it is not clear why the doubly integrated EPR signals in Figures 3-9 b,d and 3-10b have adopted negative values in some cases. There is also the question concerning working electrodes used in these experiments. Was it really ITO glass, as was

stated in Figures captions ? If so, the Author should present the construction of the EPR cells, because using ITO electrode for this purpose is very unusual and technically rather difficult.

Regarding microscopic studies, it is known that the morphology of electrodeposited polymers is strongly dependent on the resulting film thickness. Initially formed films frequently are smooth but upon increasing thickness they become more rough and often adopt more complex morphology. Thus, comparative studies of SEM images (Figure 3-6) should be performed for films of similar thickness, which apparently is not the case of pCNZ1 and pFM3.

Chapter 4.1 is devoted to the fabrication of an electrochemical sensor for selective determination of cilostazol and 3,4-dehydrocilostazol (dhCIL). The developed device had rather complicated structure, consisting of CIL–template molecularly imprinted polymer nanoparticles immobilized on the electrode surface by electropolymerized tyramine. Each step of this sensor fabrication required careful optimization. As a result relatively low detection limits for CIL and dhCIL have been achieved. In addition, the constructed device was highly selective and its performance was not disturbed by the presence of cholesterol or glucose.

In Chapter 4.2 a different approach leading to the fabrication of cilostazol chemosensor is described. In this case CIL molecules were introduced into the polymer layer during electropolymerization of functional and cross-linking monomers. The functional monomer FM1 (4-[3,6-di(thiophen-2-yl)-9*H*-carbazo-9-yl]benzoic acid) has been selected on the basis of theoretical modeling of potential complexes with the analyte. MIP films were electrochemically deposited from a mixture CIL, FM1 and the crosslinking monomer CLM (4-bis(3,6-di(thiophen-2-yl)-9H-carbazo-9-yl)benzene). Detailed characterization of thin films prepared in this manner, including their chemical constitution, is extremely difficult. The Author claims that PM-IRRAS measurements confirm "*the successful copolymerization and deposition of all monomers*". Unfortunately, the shown spectra of MIP and NIP films are of rather bad quality and cannot be treated as conclusive. The interpretation would be more convincing, if the spectra of electrodeposited films were compared with the spectra of the monomers and the pre-polymerized complexes.

Chapter 5 is devoted to the preparation of a chemosensor for duloxetine (DUL). The procedure for the DUL- MIPs fabrication was similar to that described in Chapter 4.1. First duloxetine molecules were immobilized in the polymer matrix obtained by free-radical polymerization of methacrylic acid in the presence of ethylene glycol dimethylacrylate. After template removing, the resulting nanoMIPs were immobilized on the electrode surface by

electropolimerization of tyramine. Addition of carbon nanotubes to the electrolyte mixture significantly improved the chemosensor performance.

It must be stressed, that the experimental part of the thesis distinguishes itself by the use of a large number of complementary characterization methods such as electrochemical techniques, including impedance measurements, spectroscopic (UV-vis, emission, FTIR) and spectroelectrochemical (UV-vis-NIR, EPR) methods as well as DLS and electron microscopy. In addition, quantum-chemical calculations are widely used for modeling and simulation of new molecules and MIPs structures. The discussion of the results is profound and in the majority of cases well justified by the performed experiments.

The scientific content of this dissertation is very extensive and largely sufficient for a PhD Thesis. In this plethora of results some nomenclature, editing and typing errors may however appear. Some of them are listed below:

1. The title of Chapter 4 (page 107) is identical to the title of chapter 4.1 (page 108).
2. Table 1-1 (page 11). Polymerization methods are listed but polymers used for MIP preparation are missing.
3. Some statements concerning conducting polymers are not accurate (see below).

On pages 20/21 the Author writes: *"CPs are classified as p- or n-type semiconductors, whether easily oxidized or reduced. Positive charges can be stabilized by n-type polymers with electron-rich structures, whereas p-type polymers can stabilize negative charges with electron-deficient networks"*. In reality, it is the opposite. p-type polymers are positively charged in their oxidized state, and n-type polymers are negatively charged in the reduced state. The expression, to quote *"When a polymer backbone is oxidized, π electrons of this backbone are lost"* is not correct. Oxidation of conductive polymers involves the removal of a fraction of π-electrons, the remaining π-electrons adopt the quinoid-type sequence of conjugation. And subsequently, quoting: *"Because of the removal of the π electron, this radical (polaron) is then further oxidized to a diradical (bipolaron). This benzoid to quinoid transformation is faster in the case of diradicals (a spinless quantity or bipolarons). If further oxidized, diradical (bipolaron) orbitals overlap again, creating a small band within the main band structure with the lowest energy band gap"*. The term "bipolaron" has different meaning than "diradical". Bipolaron, in chemical nomenclature, is equal to "spinless dication" or "spinless dianion", and polaron to "radical cation" or "radical anion".

4. Page 31: the caption of Figure 1-13 there is a spelling error in the name of the compounds.

5. Page 95: Figures 3-7c and 3-7d should be mentioned rather than Figures 3-4c and 3-4d.

6. Page 118: the caption of Figure 4.1-5 contains some errors. It should be: The curves of (a, b, c) CV, (d) DPV and (e) EIS ….

The points and remarks listed above are rather minor and do not lower the extremely high quality of the evaluated dissertation.

To summarize, I have no doubts that the PhD dissertation of Jyoti, M.Sc., fulfils all requirements listed in Article 187 of the Legal Act of July 20, 2018 concerning the University Education and Science (Dz. U. from 2018 poz. 1668 with later changes). Therefore, I recommend to allow Jyoti to take further steps of the conferment procedure for her doctoral degree and the public defense of her PhD dissertation.

Furthermore, taking into account extremely high quality of the presented research work, its originality and outstanding achievements in the field of elaboration of chemosensors, I am convinced that Jyoti merits a PhD degree with distinction. The research described in this dissertation gave rise to several articles, two of them already published in prestigious scientific journals. Importantly, Jyoti was the first author in all those papers, which evidenced her dominating role in the described investigations. In addition she was the co-author of several patent applications.