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Review report on the Doctoral thesis of MSc Mykola Kravets
entitled
***"Discrimination of homologous and isomeric dicarboxylic acids by gold
nanoparticle-pillar[n]pyridinium ensembles"***

The dissertation of Mykola Kravets entitled "Discrimination of homologous and isomeric dicarboxylic acids by gold nanoparticle-pillar[n]pyridinium ensembles" was prepared in the Institute of Physical Chemistry of the Polish Academy of Sciences under the supervision of Dr. Habil. Volodymyr Sashuk (Assoc. Prof. IPC PAS).

The thesis covers the problem of detection and discrimination of different types of dicarboxylic acids. Indeed, these compounds are widely used in the chemical industry. For example, they are used to prepare polymers, synthesize medicines, dyes, cosmetics and many others. In addition, they are an essential element of metabolic processes in living organisms. Not surprisingly, the development of detection methods for these compounds is significant from the industrial and medical points of view. The author of this thesis's primary goal of scientific interest was to design, prepare and test molecular sensors that can be used in sensing and discriminating dicarboxylic acids. Mykola Kravets prepared two types of gold nanoparticle-pillar[n]pyridinium ensembles to accomplish these goals. He used them in the colorimetric analysis of isomers of phthalic acid isomers, homologous series of straight-chained dicarboxylic acids and geometric isomers of dicarboxylic acids. To understand the mechanisms of interactions between nanosensors and analytes, he applied additional physicochemical techniques, including X-ray crystallography, Dynamic Light Scattering (DLS), zeta-potential measurements, infrared spectroscopy and transmission electron microscopy.

The layout of the doctoral dissertation of MSC Mykola Kravets is classic. The text of the work, 125 pages long, is divided into an abstract, a literature part, the purpose of the work and the results of his research, with a discussion and a description of the materials and methods used. The purpose

of the work is formulated correctly. The literature review and discussion of the results are supported by 141 literature references published in recognized periodicals. The literature being part of the Ph.D. thesis sufficiently describes the current state of knowledge regarding methods for discrimination of types of dicarboxylic acids, with an emphasis on: (a) isomers of phthalic acid, (b) homologous series of straight-chained dicarboxylic acids and (c) geometric isomers of dicarboxylic acids. The beginning of each paragraph introduces the reader very well to the topic presented in a particular subsection. This brief review was performed professionally based on correctly selected and current literature. The Ph.D. candidate describes numerous examples of discrimination of a specific group of dicarboxylic acids. Therefore, some summary (e.g., as a table) for each subsection would be helpful for the reader. The results section introduces the concept of supramolecular nanosensors based on gold nanoparticles and P4P or P6P, as well as its design. Next, the Ph.D. candidate describes the application of the nanosensors in recognition of the dicarboxylic acids. The text of the work is provided with 121 illustrations and 4 tables. The dissertation ends with conclusions and further perspectives for other applications of resultant nanosensors in sensing compounds containing other than anionic carboxylic groups.

The major strength of the thesis is a successful elaboration of the preparation procedure to obtain stable AuNPs-P4P and AuNPs-P6P sensors, which were further applied in the detection of a variety of dicarboxylic acid anions. In particular, the Ph.D. candidate revealed that a system based on AuNPs-P4P ensemble could be applied for sensing and discriminating all three phthalate isomers. For the detection and sensing of homologous series of straight-chained dicarboxylic acid anions, Mykola Kravets applied two sensors, namely AuNPs-P4P and AuNPs-P6P. Also, for some dicarboxylic acid anions, discrimination was achieved. Finally, successful recognition and discrimination were also confirmed for geometric isomers of dicarboxylic acids, including (2E)- or (2Z)-but-2-enedioic acid, 4,4'-(E) or (Z)-diazene-1,2-diylidibenzoic acid and stilbene-4,4'-(E)- or (Z)-dicarboxylic acid.

The weaker sides of the thesis:

1. Within the result section, the Ph.D. candidate wrongly points out that his research relates to interactions of PnP-NP with dicarboxylic acids. These statements confuse the reader, as the ensembles are formed with deprotonated dicarboxylic acids (preferably dianions). In the same section, MSc Kravets indicates that sodium salts of dicarboxylic acids were used in experiments for all three dicarboxylic acid series under investigation. Confusingly, in the experimental part, the Ph.D. candidate indicates a free form of dicarboxylic acids, not their salts, to be used in experiments related to sensing geometric isomers of dicarboxylic acids. It should be clearly stated that ionized forms of not-free dicarboxylic acids are detected and determined by the sensors within this study. The problem of protonation/deprotonation of both

carboxylic groups is raised mainly in subsection 2.5. However, without mentioning pKa1 and pKa2 values for carboxylic groups (see point below).

2. In the last part of the result section, during research on sensing geometric isomers of dicarboxylic acids, the Ph.D. candidate raises an issue of pH's influence on sensing deprotonated dicarboxylic acids. Unfortunately, this subject was omitted entirely in other experimental sections related to the sensing of phthalic acid dicarboxylic acids homologs, as information needs to be provided on the sensing environment's pH. The pH issue is fundamental, especially in the context of pKa1 and pKa2 values for carboxylic groups (as revealed for fumaric and malic acids, where too low pH values affected the sensing of dianions of these acids. The pK1 value for maleic acid is around 1.7, indicating that one carboxylic group is protonated under experimental conditions. Therefore, it can interact with only one AuNPs-PnP particle independently from steric hindrances. To conclude this part, an influence of pH on the sensing process, also in the context of ionization of dicarboxylic acids, and on the stability of the sensor should be provided at the beginning of the result section. Also, the effect of pH should be carefully checked for all analyses.
3. I can't entirely agree with the statement that P6P@AuNPs are less versatile than those based on P4P (Pages 53, 69, 81). First, experiments with P6P@AuNPs were conducted only for dicarboxylic acid homologs (within those, the effect of pH was not considered). For other series of dicarboxylic acids, the results might be different (especially when the pH of the environment is optimized). Also, zeta-potentials for P4P@AuNPs-dicarboxylic acid and P6P@AuNPs-dicarboxylic acids are similar for particular dicarboxylic acids (Table 4). Based on that, I have to disagree with the statement that P6P@AuNPs-dicarboxylic acids ensembles have lower stability than their P4P-counterparts (Page 79).
4. In the results section, the Ph.D. candidate presents the crystallographic study of terephthalate²⁻-P4P⁴⁺ salt (its salt, not a complex, as indicated in Figure 80). The crystallographic model (which can reflect interactions between AuNP-P4P and phthalates anion in solution) shows the importance of electrostatic attraction and charge-assisted hydrogen bonding. Following that, I have a few additional questions:
 - A) This study suggests that an interaction between the sensor and dicarboxylic acid anion does not require molecular cavities, as two P4P cations surround each dicarboxylic acid anion. A cavity within the P4P cation is too tiny to guest any dicarboxylic acid anion. On the other hand, the cavity is larger within P6P cation. However, as shown for the P4P salt, strong electrostatic attraction and charge-assisted hydrogen bonding occur outside the molecular cavity. Thus, the cavity might not be necessary for dianion binding. What is the opinion of the Ph.D. candidate on that?

- B) The Ph.D. candidate used one method to crystallize dicarboxylic acids²⁻-P6P⁴⁺ salts. Did he optimize it or try other techniques to obtain diffraction-quality monocrystals of those salts? Crystallographic models of dicarboxylic acids²⁻-P6P⁴⁺ salts should shed more light on that.
- C) Following the above, I speculate that the control experiment with AuNPs covered with flexible, long aliphatic chains of TMA (Pages 64 and 65) proves the crucial role of compact and rigid no-cavity molecular elements (e.g., P4P and P6p), which can enable a strong and efficient binding of phthalates. What is the opinion of the Ph.D. candidate on that?
- D) Figure 80, panel A indicates the presence of solvent molecules within the crystal lattice (green spheres). Do they correspond to water molecules? In what kind of intermolecular interactions do they participate?
5. DLS analyses; the Ph.D. candidate presents different diameters of P4P@AuNPs (page 54 - 7.7 nm, Table 3- 8.26, page 82 - 9.26 nm). These differences probably relate to various samples of P4P@AuNPs. Thus, it would be more convenient to present ranges of diameters instead of sizes for the most numerous population of particles.
 6. The Ph.D. candidate indicates that the obtained system is versatile, as it could be applied to detect other compounds which contain other than anionic carboxylic groups. Herein, a question arises if the AuNPs-PnP system is truly selective for dicarboxylic acids or could sense other compounds, e.g., phosphates or sulfonates. The same question relates to dipolar compounds, which could interact with the nanosensor charge-dipole manner. Did the Ph.D. candidate check it? What type of dicarboxylic acid samples (e.g., environmental, biological, industrial etc.) could be analyzed using the proposed nanosensor without the risk of a lack of its selectivity?

Other issues:

1. Paragraph 1.1., Page 2: Nomex is not made from isophthalic acid. It is synthesized from isophthalic chloride.
2. The quality figures, especially those included in the introduction section, could be of better quality, making interpretation/analysis difficult.
3. Numerous plots in the result section are drawn with similar color schemes within the same panels, making interpretation/analysis difficult.
4. Adding D₂O and H₂O to the list of abbreviations is unnecessary.
5. The results section, Page 85, erroneously stated that deprotonation of dicarboxylic acids prevents their interactions with NPs (it is the opposite).
6. The phrase "Dicarboxylic acids are colorless crystals with similar physicochemical properties." (Abstract section, Page XIV) is an incorrect generalization.
7. Chemical nomenclature is not based on IUPAC rules.

Despite the above remarks and comments, I have a positive opinion of the doctoral thesis of the Ph.D. candidate, as I am aware that the presented study relates to the initial stages of the project. The studies performed and presented by Mykola Kravets are ambitious and require the extraordinary skills of a doctoral student. Hence, the work submitted for assessment meets all the requirements for the doctoral thesis. The doctoral dissertation meets the conditions set out in Art. 187 of the Act of 20 July 2018 Law on Higher Education and Science (Journal of Laws of 2022, item 574, as amended). Therefore, I recommend that the Scientific Council of the Institute of Physical Chemistry of the Polish Academy of Sciences admit Mykola Kravets to further steps of the Ph.D. procedure.

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