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Review of the Ph.D. thesis „Molecularly imprinted polymers-based chemosensors for selective determination of chosen food toxins”by Viknasvarri Ayerdurai, M.Sc.

The Ph.D. thesis of Viknasvarri Ayerdurai, entitled: „Molecularly imprinted polymers-based chemosensors for selective determination of chosen food toxins,” was prepared at the Institute of Physical Chemistry, Polish Academy of Sciences, under the supervision of Professor Włodzimierz Kutner and Dr. Maciej Cieplak (auxiliary supervisor). The research group of Prof. Kutner has great experience, significant achievements, and a long track publication record in the field of molecularly imprinted polymers, covering basic studies and optimization for analytical applications. Viknasvarri Ayerdurai became an active member of this group and participated in scientific work in this novel and interesting research area.

The thesis has a traditional form and is divided into ‘Abstract’ (in English and Polish), ‘Literature Review,’ ‘Experimental’ part, and two chapters related to obtained experimental results and discussion. A summary with future perspectives is provided in Chapter 5. The whole work comprises 178 pages.

The thesis is supported by 216 references, described in a professional uniform manner (however, some titles are written using words starting with capital letters, e.g., in refs.4, 8, 9, 11, 23, etc. in contrast to the majority of references where in the titles small letters were used). Although a significant majority of references concerns papers published within the recent 20 years, some old classical and most important works were also cited. This referencing confirms that the Author can evaluate and balance the role of old (classical) and present literature.

The thesis has been written in English on an excellent and understandable level, with only single mistakes that do not distract the reader.

The thesis starts with some additional information concerning publications, patent submissions, conferences, and training activities of the Author. This work also contains a list of abbreviations and symbols, which is very useful for further reading.

At first glance, from an editorial point of view, the thesis (including the cover) looks excellent. The layout and clear and very elegant figures are helpful in reading and understanding the thesis. The way of presenting both text and graphical messages is very clear, although the described topics are sometimes complicated because of their nature. Perhaps, some figures are too complex, and therefore the main idea of the figure may not be apparent. However, this is only my personal feeling.

The Abstract of the thesis is outstanding. It describes well the topics included in the work. In the Polish version, I found two tiny mistakes in the names of chemical compounds. It would also be better to write “granica wykrywalności” instead of “limit wykrywania.”

‘Literature Review’ with ‘Research objectives’ is quite long (57 pages) but concise and well balanced. It contains the carefully selected but most important and helpful information concerning molecularly imprinted polymers (MIPs), their properties, synthesis methods, methods of their studies, and application areas, mainly in sensors. The second part of ‘Literature Review’ is devoted to food toxins studied in the ‘Experimental’ part.

I have some minor critical remarks about this part. The Author discussed various MIP systems applicable to sensors. The idea is presented in the figures attached. However, no calibration plots were included. Calibration plots are the main final and valuable results of sensor studies. Thus, it would help the reader evaluate the real applicability of described systems. The characteristic property of MIPs' chemosensors which seems quite general in many cases is the kind of analytical dependence – a decrease of the signal (e.g., DPV peak current) with the increase of the analyte concentration. In my opinion, basing analytical information on a signal decreasing with increasing concentration is not the optimal case because even some random and unexpected effects can worsen the sensor activity and thus signal decrease. The case with turn-on mode is much better - increasing signal with increasing concentration. The lack of calibration plots in the discussion makes it difficult to look at the described sensors from this perspective. However, the chapter concerning MIPs is summarized by a table (pages 39-40) presenting analytical parameters for various MIP-based sensors, which can, to some extent, compensate lack of calibration plots.

This chapter contains some minor errors, such as the lack of double 'm' in the word 'voltammetry' and the application of the word 'faradic' instead of 'faradaic.' In Scheme 1.1-5, the symbols representing the cross-linking monomer and the functional monomer are not visible. On page 27, there is incorrect information about figure numbers. Instead of Scheme 1.2-5a and 1.2-5d, there should be 1.2-6a and 1.2-6d, respectively. The information presented in Scheme 1.2-11 in point 3: "The prepared MIP … to form the electron holes, which are the main charge carriers in PPy," does not seem correct, as the main charge carriers in this conducting polymer are polarons and bipolarons. On the other hand, a short chapter concerning properties of component materials as conducting polymers in 'Literature Review' would be useful. It would be helpful for the system presented in Scheme 1.2-12 (page 35) to add information about the conductivity level.

The next chapter, 'Experimental,' comprising 40 pages, presents the chemicals used and describes the fundamentals of the experimental techniques applied, both electrochemical and non-electrochemical. Finally, the methods of MIPs synthesis and details of the experimental procedures used were described. This part is also clear and well written. It contains the necessary information, which can be helpful for a reader, e.g., a student starting work in this field.

The Author described the method of electrochemical impedance spectroscopy; however, this sub-chapter suffers from a lack of description of CPE elements (present in the equivalent circuits discussed in the next chapter concerning the results) as well as a lack of mentioning the capacitive impedimetry (CI) technique, which was the primary technique used in the determination of 7,8-DiMeIQx.

Moreover, I have some minor remarks on this part. The Author wrote (page 63): "hence, an electron from the electrode is transferred to the electroactive species when the energy of the electrons … is higher than the lowest unoccupied molecular orbital (LUMO) of an electroactive species and vice versa" – "vice versa" is incorrect because the electron is withdrawn from HOMO (not LUMO) in the opposite process.

Activities instead of concentrations should be used in the Nernst equation (equation 2.2-1, page 64). Moreover, if other reactants participate in the half-cell reaction, they should be included in the Nernst equation. In the description of symbols from Equations 2.2-9 (page 68), the concentration should be expressed in mol/mL (the unit is necessary as the equation contains a numeric factor, dependent on used units). The sentence from page 69: "Moreover, the inhibited diffusion of the electroactive species to the electrode surface….." is not clear. The number of Scheme 2.3-6 (page 71) is incorrect. It should be 2.2-6.

The next part, covering 56 pages, comprises two chapters describing experimental results for two separate systems. These results were also described in two separate publications in high-quality journals: *Bioelectrochemistry* and *J. Agric. Food Chem.* Each chapter starts with graphical abstract and is completed by a very useful summary called Conclusions.

Chapter 3 concerns electrochemical sensors selective for tyramine. Tyramine is an important compound that can serve as a biomarker of the rottenness of food products. Therefore, there is significant demand for simple methods/sensors of tyramine determination, which can be an alternative to more tedious and expensive chromatographic methods. The Author contributed to the effort for searching new sensing possibilities. This effort was successful. A highly selective chemosensor, working in the DPV or EIS mode using the hexacyanoferrate(III/II) redox probe, was developed toward that. This chemosensor was also successfully applied for tyramine determination in real samples (Mozzarella cheese).

The experiments started with electropolymerization. In this case, reporting the polymerization charge would be useful because giving only the number of cycles is not very helpful - the amount of the polymer is dependent on the flowing current. For results presented in Fig. 3.2-3 (page 106), the original DPV curves are missing. In the Sub-section 3.2.4 the Author presents the application of the EIS technique. Various equivalent circuits for MIP and NIP were applied (Fig. 3.2-11). However, there are no convincing comments concerning the selection of equivalent circuits and the role of some elements, e.g., CPE. The Author concentrated her attention on charge transfer resistance and plotted linear dependences of this resistance on analyte concentration (Fig. 3.2-11). Although the linear dependence is analytically sound, but what is the physical sense of this dependence? What is the physical sense of the slope value? The scale used for Z’ and –Z” on Nyquist plots should be the same, actually the unit for –Z” is slightly (80 - 90%) shorter than that for Z’ (however, this difference is of negligible significance).

The statistical analysis of results (including error bars) used is inconsistent. The statistical analysis would exceptionally be useful to analyze the data in Fig. 3.2-12 to confirm whether the difference in results of fitting the experimental data using various theoretical dependences is statistically significant. Table 3.2.-4 reports equilibrium constants for adsorption isotherms. However, these constants should instead be used without units (what is the sense of logarithm of the unit “mM” by calculating, e.g., ΔG^0 from the equilibrium constant?). In dimensionless equilibrium constants the concentrations of soluble compounds are usually expressed as the ratio of the actual concentration (in mol/L) and the standard concentration. As the standard concentration 1 mol/L is used. Thus, under these conditions concentrations are expressed as dimensionless data and thus the equilibrium constant is dimensionless as well. Is the identical slope of 11.67 (page 123) for two plots (in the absence and presence. Mozzarella cheese) in Fig. 3.2-12a correct?

I also have some other minor comments on this Chapter. On page 113, the decrease of nitrogen signal from XPS points to "tyramine removal or/and tetrabutylammonium cation entrapment" (or removal, as stated a few lines later)? On page 116, Fig. 3.2-b' is mentioned instead of 3.2-8b'.

Chapter 4 concerns electrochemical sensors selective for an example of heterocyclic aromatic amine (HAA): (7,8-DiMeIQx). The significance of this compound is related to its easy production during thermal treatment of meat and the carcinogenic influence of HAA. The Author proposed an innovative concept of nucleobase-functionalized MIP as a chemosensor recognition unit. This sensing method can be an excellent alternative to the expensive and time-consuming HPLC using method, and the developed chemosensor appeared selective to common biological interferences. In this case, the DPV technique did not provide analytically useful results. The optimal method used in this case was that involving capacitive impedimetry. An exciting result was obtained using this technique, which is also essential from a physicochemical point of view. It was found that different forms of the signal dependence on the analyte concentration are obtained under steady-state solution or FIA conditions. In the former, the signal decreases with concentration, while in the latter case, the signal increases. The Author found the dependence observed under steady-state solution conditions superior and used it for HAA determination in real samples. However, in this case, the signal decreases with concentration. In my opinion, the signal increase (*turn-on* mode observed under FIA conditions) seems more valuable from the analytical point of view.

The different behavior observed under steady-state solution or FIA conditions was convincingly explained by invoking different rates of the two processes. The faster process presumably involves HAA binding on the film surface (and is observed under FIA conditions), while the slower process (observed under steady-state solution conditions) involves attaining the partition equilibrium coupled with the incorporation of the analyte to imprinted cavities. This process is certainly selective, as shown in Fig. 4.2-14c. It is a pity that similar selectivity studies were not reported for determinations performed under the FIA conditions. Most likely, the selectivity would be smaller due to only the surface binding of the analyte.

I also have some other minor remarks about this Chapter. In Table 4.2-1, ΔG values are reported. Since ΔG generally depends on reactants concentrations, the standard value (independent of concentrations), ΔG^0, should be included instead of ΔG. Otherwise, the conditions (e.g. concentrations) should be specified. There are also some mistakes with figure numbering. On page 136, Fig. 4.2-4d (does not exist), 4.2-5a, and 4.2-5b were mentioned incorrectly. Fig. 4.3-12 (page 147) should be numbered as 4.2-12. What is the analyte concentration for the results presented in Fig. 4.2-15b (page 151)?

The title of Chapter 5: "Summary and future perspectives," is slightly misleading as it is a good and very useful summary of the results. However, future perspectives are discussed only in the last two paragraphs.

The obtained results were already published in two papers. According to the attached information, two other manuscripts were submitted or prepared. Also, one patent application was submitted. The results were also shown in presentations (oral or poster) at 11 conference.

Summarizing, I highly appreciate the thesis, the obtained results, and the extended discussion. Obtaining selective and reliable chemosensors, applicable also to real samples, required hard work and efforts concerning synthesis, application of many instrumental techniques, theoretical calculations, electrochemical measurements, and, of course, appropriate and critical analysis of results. These effects would not be possible without the activity of the Author, her knowledge, and experience in various areas of chemistry and laboratory techniques. The thesis is well organized, considering the volume/contribution of the main parts: 'Literature Review,' 'Experimental,' and 'Results and discussion.' Some mistakes, inconsistency, or lack of information are of minor significance and do not affect my very good opinion about this thesis.

Therefore, taking into account a large amount of work done, convincing discussion of results, obtaining selective sensors applicable to real samples, and the very nice appearance of the thesis, I have no doubts about the high value of this work, and I congratulate the Candidate to her contribution to the field of MIP chemosensors.

Therefore, I am convinced that the thesis, entitled: "Molecularly imprinted polymers-based chemosensors for selective determination of chosen food toxins," submitted by Viknasvarri Ayerdurai, fulfills the criteria mentioned in Article 187 of the Act dated 20 July 2018 (Dz. U. from 2018, poz. 1668 with changes), and can be the subject of further steps of the procedure leading to obtaining the Ph.D. degree.

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