Molecularly imprinted polymers for biomimetic sensors

Summary

The thesis comprises the following ***chapters***:

1. Introduction and the description of the doctoral topic, the used methods and concepts;
2. Articles in extension;
3. General conclusions;
4. Original contributions;
5. Dissemination;
6. References.

Keywords: molecularly imprinted polymers, sensors, sol-gel, electropolymerization, thin films

The first chapter of the thesis is structured in two parts. In the first part, a literature study is presented, referring to molecularly imprinted polymers (synthesis, characteristics, applications, etc.). In the second part, the aim and the objectives of the doctoral thesis are presented, but also the used concepts and methods.

The doctoral thesis was aimed at synthesizing and characterizing molecularly imprinted polymers films, with the end of being used for sensors development. Different synthesis methods have been studied, in order to choose the most convenient and efficient one. The synthesized films can be used for illicit drug detection (Ephedrine) and for bacteria detection (LPS from Gram- Negative bacteria – *Pseudomonas Aeruginosa*), respectively.

The thesis had the following objectives:

Objective 1: the synthesis and characterization of the MIP films by different techniques, in order to study the behavior and performance of the obtained materials, for ephedrine detection.

Objective 2: the synthesis and characterization of MIP films by sol-gel polymerization, to be used for electrochemical detection of LPS from *Pseudomonas Aeruginosa*.

In order to achieve the established objectives, the films were synthesized by different methods in order to choose the films with the best detection features.

Objective 1 was accomplished using three methods for the MIP film synthesis and by applying several characterization methods, as follows:

- Wet-phase inversion – molecularly imprinted membranes for ephedrine retention;
- Electropolymerization – molecularly organic imprinted layers for ephedrine detection;
- Sol-gel method – molecularly inorganic imprinted films for ephedrine detection;
  - Characterization methods of MIP membranes and films:
- The copolymer solutions were rheologically characterized and the membranes were investigated by different techniques, including: FT-IR, TGA, rebinding experiments;
- Electrochemical behavior of MIP films obtained by electropolymerization was investigated using cyclic voltammetry;
- The MIP films obtained by sol-gel were analyzed by different techniques: ellipsometry – for determining the optical properties and thickness, SEM, AFM, digital microscopy, FT-IR, TGA, rebinding experiments;
- The electrochemical behavior of obtained MIP films by the sol-gel method and deposited on carbon screen-printed electrodes, was investigated using cyclic voltammetry.

The *first concept* refers to the preparation of MIP membranes by wet phase inversion. The process involves the coagulation of the copolymer solution, in a non-solvent bath. The precursor solution was obtained by dissolving the copolymer and the template in a suitable solvent (at room temperature or by heating). Two synthesized acrylonitrile copolymers were used for membrane synthesis. These two copolymers were obtained by emulsion polymerization. The precursor solutions were obtained by dissolving the copolymers in dimethylformamide – DMF. After, the solutions were casted on glass substrate and immersed in the coagulation bath, which consists of distilled water. The last step of the process involved a drying process. Two pairs of membranes were obtained (each time a molecularly imprinted and a non imprinted one), using two synthesized copolymers. All membranes were characterized after the drying process. The ephedrine was extracted by washing with ethanol.

The molecular recognition step consisted in contacting the membranes with an ephedrine solution for 180 min, after the removal of template molecule.

The *second concept* refers to the synthesis of MIP films by electropolymerization. In this regard, a monomer synthesized in laboratory, namely (2-(3,4-Ethylenedioxythienyl)-3-(carboxymethylsulfanyl) thiophene and a commercial one 2,2' bithiophene were used. The MIP films synthesis was performed by electropolymerization in potentiodynamic and in potentiostatic conditions in presence and absence of ephedrine. The synthesized films were electrochemically tested before and after the ephedrine removal and after the contact with ephedrine solution.

The *third concept* refers to the synthesis of MIP films by sol-gel method. In this regard, a commercial monomer, i.e. (N-(2-aminoethyl)-3-aminopropyltrimethoxysilane) was used. The synthesis was performed under basic conditions (NH_4OH), in the presence or in the absence of ephedrine. Two sets of films were obtained, using different concentration of monomer and ephedrine. The sol-gel precursor obtained was deposited by spraying onto glass support and then dried to obtain the films. The synthesized films were analyzed before and after the template removal. The template was removed by several washing cycles with ethanol. The rebinding experiments were performed, after the template removal, by contacting the MIP and NIP films with ephedrine solution.

Objective 2 was achieved by using a fourth concept, which provides for the combination of a classical method of film preparation combined with an electrochemical detection surface:

- Sol-gel method – molecularly imprinted films with LPS from *Pseudomonas Aeruginosa*;
- Electrochemical detection – cyclic voltammetry.

The fourth concept refers to the synthesis of molecularly imprinted films by the sol-gel method, further used for the electrochemical detection of LPS from *Pseudomonas Aeruginosa.* In this regard, the sol-gel precursor solution was deposited onto working electrode of a screen-printed carbon electrodes (SPCE). In order to use the sensors for electrochemical detection, it was necessary to incorporate a doping agent. The electrochemical behavior of the films was studied before and after the LPS extraction, after the contact with LPS from *Pseudomonas Aeruginosa* solution. The selectivity of the sensors was studied by contacting the films with LPS from *Escherichia coli*. The electrochemical behavior was studied using cyclic voltammetry.

The second chapter of the thesis brings to the fore the published articles, in extenso, as a result of exploiting the obtained scientific results. The articles are:

1. **E.B. (Georgescu) Stoica**, A.M. (Florea) Gavrila, T.V. Iordache, A. Sarbu, H. Iovu, T. Sandu, H. Brisset, Molecularly imprinted membrane obtained via wet phase inversion for ephedrine retention, **U.P.B. Sci. Bull.**, Series B, 2020, 82(2), pp. 15-26.

2. **B.E. Georgescu, C. Branger**, T.V. Iordache, H. Iovu, O.B. Vitrik, A.V. Dyshlyuk, A. Sarbu, H. Brisset, Application of unusual on/off electrochemical properties of a molecularly imprinted polymer based on an EDOT– thiophene precursor for the detection of ephedrine, **Electrochemistry Communications**, 2018, 94, pp. 45-48.

3. **E.B. Stoica**, A.M. Gavrila, C. Branger, H. Brisset, A.V. Dyshlyuk, O.B. Vitrik, H. Iovu, A. Miron, A. Sarbu, T.V. Iordache, Evaluation of Molecularly Imprinted Thin Films for Ephedrine recognition, **Materiale Plastice**, 2019, 56, 4, pp. 865-874.

4. **B.E. Stoica**, A.M. Gavrila, A. Sarbu, H. Iovu, H. Brisset, A. Miron, T.V. Iordache, Uncovering the behavior of screen-printed carbon electrodes modified with polymers molecularly imprinted with lipopolysaccharide, **Electrochemistry communications**, 2021, 124, 106965.

The third chapter sums up the general conclusions of the doctoral thesis.

The doctoral thesis was aimed at synthesizing and characterizing molecularly imprinted films. As it was presented in second chapter, the thesis relies on four paper articles.

- In first study, molecularly imprinted membraned with ephedrine were prepared by wet phase inversion, in order to be used for ephedrine detection from aqueous solutions. The membranes were synthesized using two copolymers with different concentrations (C3 - 80% AN and 20% AM, C4 - 75% AN and 25% AM), thus two sets were obtained (C3-NIP/C3-MIP and C4-NIP/C4-MIP). Thermal analyzes showed a higher thermal stability for the membrane based on C3 copolymer compared to the membranes prepared using C4 copolymer. After ephedrine removal, both sets of membranes underwent re-binding experiments to determine the imprinting factor. The C3 set presented superior recognition and re-binding properties compared to C4 set. The imprinting factor determined for C3 set was 3,06 after 30 minutes, as compared to C4 set in which case a value of 1,8 was recorded after 180 minutes. It can be concluded that C3 membranes showed a higher thermal stability compared to C4 membranes, but the most important observation referred to the fact that C3 membranes were able to fast recognize and re-bind the ephedrine molecule.

- *In the second study,* molecularly imprinted films were synthesized by electropolymerization for ephedrine electrochemical detection. The films were synthesized under potentiostatic and potentiodynamic conditions, in the presence and in the absence of ephedrine, in order to establish the optimal conditions. The electrochemical behavior of the films was studied before and after the ephedrine removal from the imprinting cavities, but also after the contact with ephedrine solution in the re-binding process. The experiments revealed considerable differences between cyclic voltammograms. Following the ephedrine removal process, cyclic voltammograms highlighted the disappearance of the anodic and cathodic peaks in case of MIP films, which can be attributed to the ephedrine removal, compared to NIP films, in which case the washing procedure led only to a slight decrease in the current intensity. After the first washing procedure, the films were contacted with the ephedrine solution and the reappearance of the anodic peak occurred. The films were washed for the second time with water in order to remove the adsorbed ephedrine and, again, a disappearance of the anodic peak was registered. After the second contact with the ephedrine solution, the anodic peak characteristic for ephedrine reappeared. The obtained results were promising for the development of electrochemical sensors with MIP films, for ephedrine detection.

- *The third study* presents the synthesis of molecularly imprinted films with ephedrine, by sol-gel method. The preparation of the precursor solution was carried out by mixing the solution containing the monomer and the template dissolved in ethanol, together with the catalytic medium, for two hours. The films were synthesized using different concentrations of monomer and ephedrine, noted as diluted (D) and concentrated (C). The films were deposited by spraying the precursor solution on glass slides. After deposition, the films were dried for 48 hours at room temperature and 48 hours in the oven at 80 ^{0}C. All films were analyzed by different methods to determine their physical-chemical properties and their ability to recognize and rebind ephedrine from aqueous solutions. The optical properties showed that the film thickness is influenced by the monomer concentration: the films were thinner when lower concentration of monomers was used. On the other hand, films obtained from solutions with lower monomer concentration were more homogeneous, being less rough, compared to those obtained from solutions with higher monomer concentration. The re-binding experiments performed on MIP and NIP D pair, led to important values of the imprinting factors (5.7, 4.58, 5.8, 6.2 and 5.8 at 1, 5, 10, 15 and 20 min, respectively, after contact with the ephedrine solution), confirming the efficiency of the imprinting process. Given the results, the films synthesized at lower monomer concentration were more suited for the development of sensors.

- *The fourth study* describes the synthesis of LPS imprinted films, using LPS from *Pseudomonas Aeruginosa*. The films were synthesized by a sol-gel method and their deposition was made by dripping the precursor solution onto screen printed carbon electrodes. In order to use the films for electrochemical detection, it was necessary to use a doping agent (ZnO) to increase the conductivity. For the synthesis of the films, a lower concentration of monomer was chosen, compared to the study that was the subject of the previous research. All the films were electrochemically tested before and after LPS extraction, and after contact with LPS from *Pseudomonas Aeruginosa* (for specificity testing). The selectivity tests consisted in contacting the films, after the second washing procedure, with an LPS solution from *E. coli*. Considering the electrochemical tests, the resulting voltammograms showed substantial differences between MIP and NIP films before LPS extraction, after LPS removal and after contact with LPS solutions. The obtained results are promising for the development of a detection method based on molecularly imprinted polymers with LPS from *Pseudomonas Aeruginosa*.

The fourth chapter refers to original contributions of the doctoral thesis. As it was previously mentioned, the doctoral thesis is based on scientific results published in four paper articles.

1. An original method for the synthesis of MIP membranes by wet phase inversion was developed. The C3 (80% AN, 20% AM) membranes were able to recognize and to re-

bind ephedrine from aqueous solution, faster than C4 (75% AN, 25% AM) membranes, as it was shown by the imprinting factor.

2. An original method for molecularly imprinted films synthesis by electropolymerization was developed. The originality of the study was the electropolymerization system used. The choice of monomer was based on its ability to form bonds with ephedrine, which possesses secondary amine groups. The differences in the recorded voltammograms of MIP and NIP films, after electropolymerization, ephedrine removal/washing and contact with ephedrine, confirmed the success of the imprinting process. The results are hence promising for developing a procedure for producing sensors that can be used for ephedrine detection.

3. An original method for molecularly imprinted films synthesis by sol-gel method was developed. Ellipsometry, used to determine the optical properties, showed that the films were thinner at lower monomer concentration, as compared to the films obtained at higher concentrations. At the same time, the ellipsometry also highlighted the fact that the transmission and the refractive index were influenced by the monomer concentration. Thermogravimetric analysis showed a high thermal stability. The rebinding experiments highlighted a rapid response of films at contact with the ephedrine solution, which resulted from the registered values of the imprinting factors (5.7, 4.58, 5.8, 6.2 and 5.8 at 1, 5, 10, 15 and 20 min, respectively). The resulted imprinting factors confirmed that specific imprinted cavities for ephedrine were formed. The method developed to synthesize the MIP films has multiple advantages in addition to simplicity, in terms of low costs and fast re-binding. Thus, prepared MIP films present potential for application in the field of optical sensors due to their suited optical characteristics.

4. An original method for molecularly imprinted films synthesis with LPS by sol-gel method was developed. According to the literature, this study represents the first attempt to obtain biosensors for LPS detection. TEM analysis highlighted the self-assembly mechanism between the monomer and LPS molecules in the pre-imprinting step. The synthesis process was simple, i.e., short reaction time, simple deposition method and room temperature conditions. The use of screen-printed carbon electrodes represented another advantage of the proposed concept due to the low costs, availability and small size, which leads to the use of

small amounts of precursor solution for modifying the carbon surface but also to the use of small amounts of sample for detection. Following the tests, the films showed a fast recognition time, selectivity and reusability. Given all these advantages, the proposed concept presents high interest for sensors development.

The fifth chapter presents the scientific results published in the doctoral thesis.

Scientific ISI Articles

1. E.B. (Georgescu) Stoica, A.M. (Florea) Gavrila, T.V. Iordache, A. Sarbu, H. Iovu, T. Sandu, H. Brisset, Molecularly imprinted membrane obtained via wet phase inversion for ephedrine retention, U.P.B. Sci. Bull., Series B, 2020, 82(2), pp. 15-26.

2. B.E. Georgescu, C. Branger, T.V. Iordache, H. Iovu, O.B. Vitrik, A.V. Dyshlyuk, A. Sarbu, H. Brisset, Application of unusual on/off electrochemical properties of a molecularly imprinted polymer based on an EDOT– thiophene precursor for the detection of ephedrine, Electrochemistry Communications, 2018, 94, pp. 45-48.

3. E.B. Stoica, A.M. Gavrila, C. Branger, H. Brisset, A.V. Dyshlyuk, O.B. Vitrik, H. Iovu, A. Miron, A. Sarbu, T.V. Iordache, Evaluation of Molecularly Imprinted Thin Films for Ephedrine recognition, Materiale Plastice, 2019, 56, 4, pp. 865-874.

4. B.E. Stoica, A.M. Gavrila, A. Sarbu, H. Iovu, H. Brisset, A. Miron, T.V. Iordache, Uncovering the behavior of screen-printed carbon electrodes modified with polymers molecularly imprinted with lipopolysaccharide, Electrochemistry communications, 2021, 124, 106965.

Comunications to International Conferences

1. E.B. Stoica, A.M. Gavrila, T. Sandu, L. Ciurlica, H. Brisset, C. Branger, H. Iovu, A. Sarbu, A.L. Radu, A. Zaharia, T.v. Iordache, Molecularly imprinted polymer films doped with conductive substances for bacteria detection, 8th Graduate Student Symposium on Molecular Imprinting, 28-30 August 2019, Berlin, Germany. Poster

2. E.B. Stoica (Georgescu), H Brisset, C. Branger, H. Iovu, A.M. Florea, A.L. Radu, T. Sandu, A. Zaharia, A. Sârbu, T.V. Iordache, Preparation of a new molecularly imprinted

polymers via electropolymerization for ephedrine detection, 6thNanoToday Conference, 16-20 June 2019, Lisbon, Portugal. Poster

3. E.B Stoica, A.M. Gavrila, A. Sarbu, A. Miron, M. Ghiurea, B. Trica, V. Raditoiu, R. Botez, I. E. Neblea, T.V. Iordache, Electrochemical Sensor Based on Molecularly Imprinted Polymers for Lipopolisaccharides Detection, PRIORITIES OF CHEMISTRY FOR A SUSTAINABLE DEVELOPMENT" PRIOCHEM – XVIth Edition, 28-30 October 2020, Bucharest, Romania. Oral Communication.

Patent applications and awarded patents

1. A. Sarbu, T.V. Iordache, A.M. Florea, E.B. Georgescu, S. Apostol, "Filme polimerice imprentate molecular cu droguri şi procedeu de obţinere a acestora/ Molecularly imprinted polymer films with illicit drugs and a process for obtaining thereof", Patent application A 00920/09.11.2017 Patent RO **133363B1/2021**

2. T.V. Iordache, E.B. Stoica, A. Sârbu, A.M. Gavrilă, A.L. Ciurlică, A.L. Chiriac, A. Zaharia, T. Sandu, „Suprafețe hibride pentru detecția electrochimică a endotoxinelor microbiene și procedeu pentru obținerea acestora/ Hybrid surfaces for electrochemical detection of microbial endotoxins and a process for obtaining thereof", Patent application A00804/27.11.2019