Development of a Molecularly Imprinted Sensing Material for Antibiotics Detection

Hugues Charlier[1], *Sébastien Hoyas*[2], *Vincent Lemaur*[2], *Driss Lahem*[3], *Jérôme Cornil*[2] , *Marc Debliquy*[1]

[1] *Material Sciences Department, UMONS, Mons B-7000, Belgium,*
[2] *Laboratory for Chemistry of Novel Materials, UMONS, Mons B-7000, Belgium,*
[3] *Materia Nova, Materials R&D Center,* Mons B-7000*, Belgium*

Corresponding Author: Hugues Charlier
Email adress: hugues.charlier@umons.ac.be
Adress: Rue de l'Epargne 56, B-7000 Mons (Belgium)
Phone number: +32 65 37 44 30
Sébastien Hoyas: sebastien.hoyas@umons.ac.be
Vincent Lemaur: vincent.lemaur@umons.ac.be
Driss Lahem: driss.lahem@materianova.be
Jérôme Cornil: jerome.cornil@umons.ac.be
Marc Debliquy: marc.debliquy@umons.ac.be

Introduction

Antibiotic detection has become a major issue in many fields such as water analysis, food control, health, etc. Besides the immediate dangers, the spread of antibiotics everywhere provokes the phenomenon of bacterial resistance which, on the long run, will cause health issues. Indeed, the excessive use of antibiotics allows the bacteria to mutate and thus develop resistance, requiring the development of new compounds.

This work aims to develop and characterize a sensing material to detect multiple antibiotics in liquid phase using various methods. The idea is to conceive low power sensors or even disposable sensors. Therefore, sensors should be low cost and compatible for mass production. The most interesting sensitive materials appear to be Molecularly Imprinted Polymers (also called MIP) using conductive polymers [1],[2].

Synthesis of the MIP

Polypyrrole (PPy) is chosen as conductive polymer to be molecularly imprinted. Pyrrole (Py) is used as the cross-linker and pyrrole-3-carboxylic acid as the functional monomer, to obtain chemical interactions between the polymer and the target molecule (also called "template"). The synthesis is performed in-situ (also called bulk polymerization) in aqueous media by mixing the target molecule, the functional monomer and the cross-linker with the oxidizer, ammonium persulfate (APS), while the pH value is set to around 2.2, well below the template pKa to allow better interactions between monomers and the template. During oxidative polymerization, the temperature is kept at 30 °C, for 2 hours. The polymer is then deposited on the substrates used to get the sensor.

The next step, so-called extraction, consists in removing the template from the polymer. It is performed by using a solution of methanol and HCl (9:1) wherein substrates are immersed for 2 hours [3]. Then, substrates are stored in PBS (phosphate buffered saline) solution before being tested. Non-Imprinted Polymers (NIP) are also synthetized following the same steps, except that the template is not present during the synthesis.

Measurement methods

Polymerization has been performed on various substrates allowing to use multiple sensing methods to explore as much paths as possible. Sensitive materials have been deposited on chemoresistive plastic IDE (InterDigitated Electrodes) to perform EIS measurements (Electrochemical Impedance Spectroscopy), on quartz crystal microbalance to perform mass measurements and on glass slide to perform optical absorbance measurements, as shown on Fig.1.

Results and Conclusions

After the extraction step carried out on the various substrates, detection is performed by adding the target antibiotic into the PBS solution. Fig. 2 shows impedance measurements revealing that this parameter grows nearly linearly as soon as we add a dozen ppb of the antibiotic. Fig.3 shows that using the same measurement method, the NIP does nearly not react when we add the template, which means that the imprinting is functional and significantly increases the performance of the sensor. Similar results were obtained with spectrophotometry NIR (Near InfraRed)measurements on Fig. 4 and Fig. 5. This method exhibits some limitations with a saturation above 50 ppb.

Beside these practical measurements, modeling has been performed by MD (Molecular Dynamic) to simulate 2 steps of the practical process: the pre-polymerization assembly and the extraction step. EIS fittings have also been achieved on the experimental results. An example of these fittings is presented on Fig 6, where it is possible to observe the good correlation between the fitting and the experimental curves, at 0 ppb for sensitive layers synthesized at various pH values. The utilization of fitting and modeling software aims to provide a better understanding of the physical phenomena occurring during the experiments.

In conclusion, the sensors were found to be able to detect concentrations of approximately 15 ppb and are close to the detection limits of conventional methods (10 ppb). In addition, they have proven their specificity to the target molecule by showing no reaction when exposed to the usual interferents. We showed that the MIP is suitable for both impedimetric sensors as well as for optical sensors (optical fibers or dipsticks for instance).

References

[1] S. A.Piletsky, N. W.Turner, P. Laitenberger, Molecularly imprinted polymers in clinical diagnostics – future potential and existing problems, Medical Engineering & Physics , 28 (2006), 971-977. doi:10.1016/j.medengphy.2006.05.004

[2] M. Díaz-Bao, R. Barreiro, J. M. Miranda, A. Cepeda et P. Regal, Fast HPLC-MS/MS Method for Determining Penicillin Antibiotics in Infant Formulas Using Molecularly Imprinted Solid-Phase Extraction, Journal of Analytical Methods in Chemistry, 2015 (2015). doi: 10.1155/2015/959675

[3] J. L. Urraca, A. J. Hall, M. C. Moreno-Bondi, B. Sellergren, A Stoichiometric Molecularly Imprinted Polymer for the Class-Selective Recognition of Antibiotics in Aqueous Media, Angewandte Chemie, 118 (2006), 5282-5285. doi: 10.1002/ange.200601636

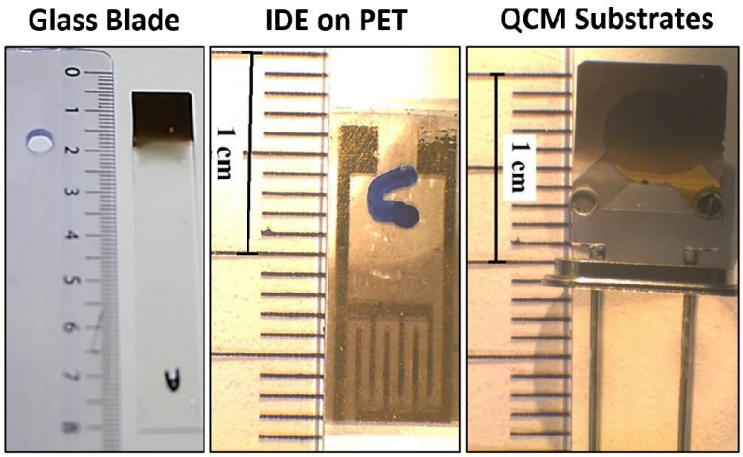

Fig. 1 Substrates used to déposit the sensitive material

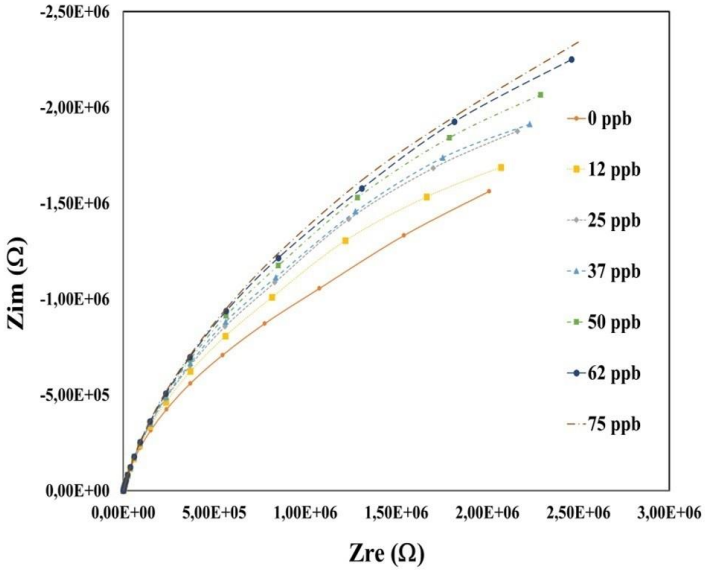

Fig. 2 MIP impedance variation while adding the antibiotic

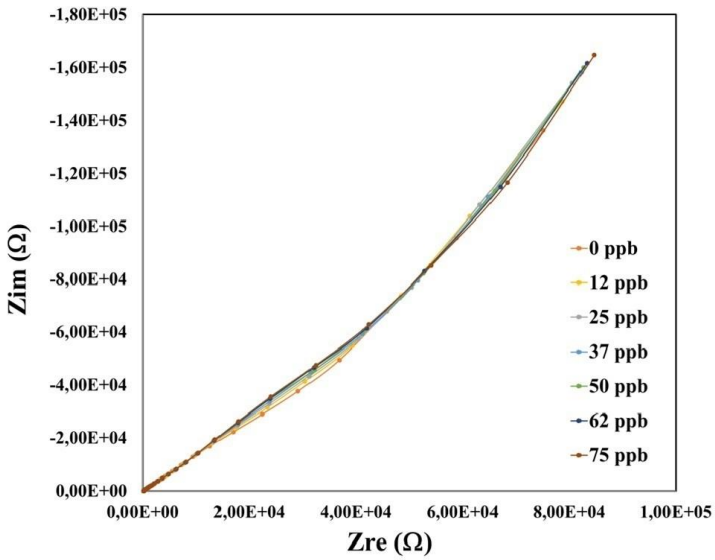

Fig. 3 NIP impedance variation while adding the antibiotic

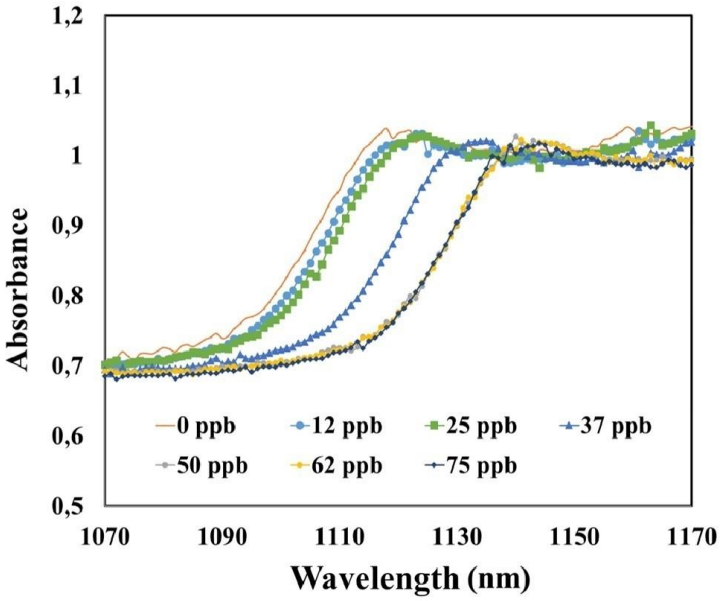

Fig. 4 MIP absorbance variation while adding the antibiotic

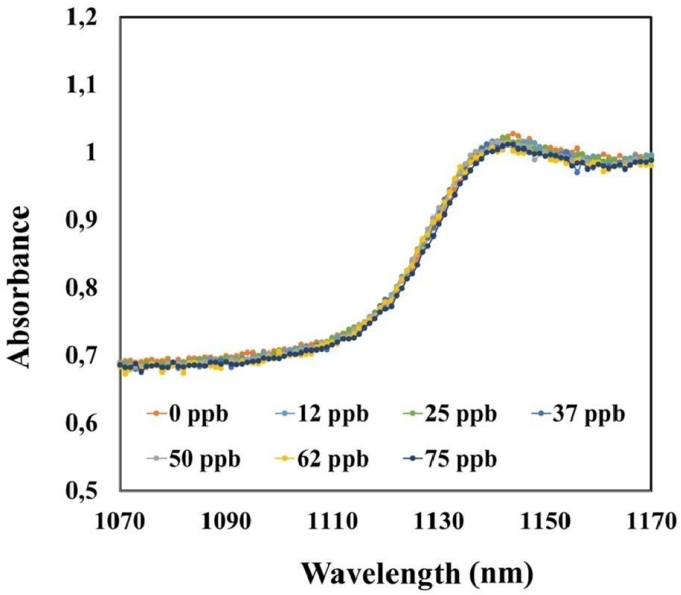

Fig. 5 NIP absorbance variation while adding the antibiotic

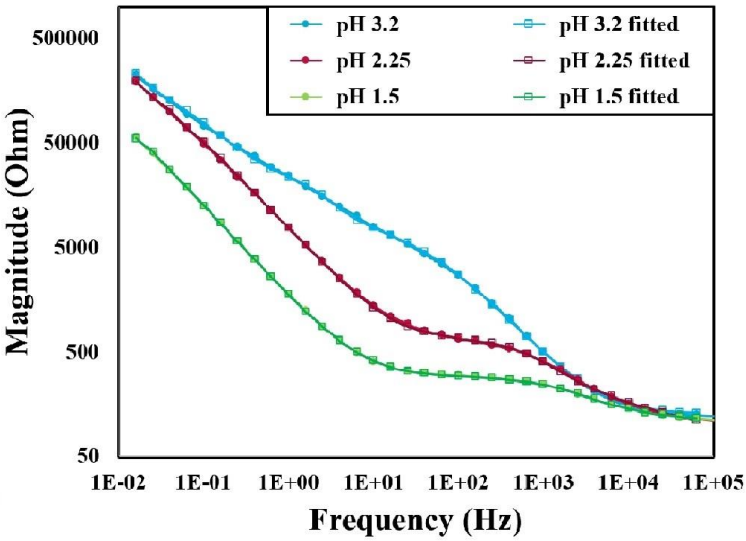

Fig. 6 EIS fitting on sensitive MIP layers synthesized at different pH values before the detection (at o ppb)