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## EUROSENSORS 2015

## Surface acoustic wave sensor for selective detection of flumequine

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**Abstract**

A surface acoustic wave (SAW) sensor functionalized with a thin layer of polypyrrole (PPy) molecularly imprinted polymer (MIP) was designed for the selective detection of flumequine (FLU), a fluoroquinolone antibiotic usually used in intensive aquaculture. Contrarily to previous studies, in which immunosensors were developed to detect low levels of fluoroquinolone, here, the recognition layer does not require any special treatment for stability over a long period of time. The detection limit was of order of  $10^{-6}$  M and the sensitivity was estimated at  $9.4 \pm 0.4^\circ/\text{mM}$ . Selectivity tests, made with levofloxacin, show that the developed sensor displayed an excellent recognition capacity toward flumequine. Quantum chemical calculations, based on the density functional theory (DFT), have permitted to highlight the nature of interactions between the investigated molecular systems and polypyrrole matrix.

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**Keywords:** SAW sensor; Molecularly imprinted polymer (MIP); Flumequine (FLU); Polypyrrole (PPy); Levofloxacin (Levo); DFT calculations

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## 1. Introduction

It is believed that aquaculture will be the largest source of seafood consumed in the world in the coming years [1]. However, aquaculture expansion may be unsustainable as it generates unwanted effects such as contamination of the environment with chemicals and antimicrobial. In addition to the serious environmental problems, residues of antibiotics are a real danger to human health since their abusive use leads to the development of antibiotic-resistant bacteria and allergic reactions [2]. Among the commonly antibiotics, fluoroquinolones, in general, and flumequine (FLU), in particular, are the most used drugs for fish treatment in aquaculture ponds [3].

Owing to their extensive use, the residuals of the fluoroquinolone antibiotics may remain not only in fish tissues, but also in bass water. Nevertheless, there is a lack of legislation regarding tolerance levels of this veterinary drug in natural waters. To our knowledge, there are no recommended limits for antibiotics in aquatic environments.

A large number of analytical methods have been proposed for the analysis of quinolones in food: chromatographic techniques [4], microbiological assays [5], ELISA [6] and immunosensors [7]. Although their inherent advantages, the high cost, lack of selectivity and poor physico-chemical stability of the antibodies or enzymes prevent their use in routine analysis in aquatic environments.

In the present study, we report the use of a molecularly imprinted based surface acoustic wave (MIP-SAW) sensor for flumequine detection. The FLU recognition layer is an electropolymerized polypyrrole MIP. This choice was motivated by the fact that these materials are easy to prepare, stable, inexpensive, selective, and can be used in highly charged media like those of aquaculture ponds [8-9]. The combination of molecular imprinting and gravimetric transduction is consequently an attractive route to enhance both selectivity and sensitivity of a considered chemsensor.

To our knowledge, this is the first study concerning flumequine detection in water media with a chemsensor in general, and a MIP-SAW device in particular.

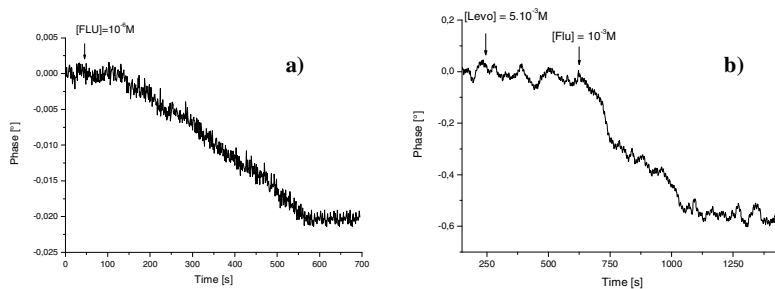
## 2. Details of MIP-SAW sensor

The developed SAW sensor consists of a dual delay lines fabricated on a  $36^\circ$  YX-LiTaO<sub>3</sub> piezoelectric substrate with an operating frequency of about 104 MHz. The measurement setup consists of a functionalized SAW sensor, a Kalrez® flow cell, a PMMA sill including inlets and outlets connected to a peristaltic pump, and a homemade pulse measurement system [10]. Electrochemical measurements were carried out using portable PG581 potentiostat apparatus interfaced with PG581 software. A conventional three-electrodes system was used with a steel grid as auxiliary electrode, an Ag/AgCl electrode as the reference one, the working electrode was the SAW sensing area ( $S = 22 \text{ mm}^2$ ). All experiments were carried out at room temperature.

MIPs electropolymerization was carried out by chronoamperometry (CA) using a  $10^{-2}$  M FLU template solution, which was in  $10^{-1}$  M tetrabutyl ammonium tetrafluoroborate (tBuNBF<sub>4</sub>) and in  $10^{-1}$  M pyrrole. A very thin PPy layer was firstly electropolymerized by CA during 2 s on the SAW sensing area, before starting MIP growth on the electrode surface, to avoid FLU complexes' formation on the SAW surface [11]. Non-imprinted polymers (NIPs) film have also been prepared under the same electrodeposition conditions but without any FLU.

## 3. Results and discussion

During the electropolymerization process, FLU molecules are trapped in the vicinity of the polymer due to the formation of hydrogen bonds between FLU and pyrrole. Therefore, for FLU molecules extraction, a continuous flow ( $0.19 \text{ mL min}^{-1}$ ) of a protic solution (MeOH/AcCOOH: 90/10 V/V) was pumped over the sensing area of the SAW sensor. An increase of phase values versus time indicated that FLU molecules were removed from the MIP matrix by breaking the hydrogen bonds (results not shown here). After that, the MIP was rinsed with a solution of NaCl 0.1 M adjusted to pH 8 with a solution of NaOH to approximate aquaculture ponds conditions. Phase variations versus time after the injection of FLU at  $10^{-6}$  M and at  $10^{-3}$  M are plotted in Figs. 1.a and 1.b respectively.



**Fig.1:** Phase variation versus time after: (a) injection of a solution of flumequine  $10^{-6}$  M  
(b) injection of solutions of levofloxacin  $5.10^{-3}$  M then flumequine  $10^{-3}$  M.

In both cases, FLU injection leads to a phase decrease, indicating that the PPy-MIP cavities recognized the analytes. The main difference between the two graphs, besides the phase shift amplitude, is related to the kinetic of the recognition which is slow for low concentrations and relatively fast for high ones. This difference is probably related to the diffusion rate of the analytes, from the solution to the surface sensing area, which is concentration-dependent. Sensitivity of the developed MIP-SAW sensor, calculated from the slope of the phase/concentration curve, was found equal  $9.4 \pm 0.4^\circ/\text{mM}$ .

Selectivity tests were made with levofloxacin (Levo), an interfering fluoroquinolone antibiotic equally used in aquaculture ponds. Fig. 1b shows that the injection of a solution of  $5.10^{-3}$  M of Levo does not cause any phase shift. The oscillations, following this injection, are probably due to instable bonds, as the molecular structure of Levo does not perfectly match to the FLU imprints.

Effects of nonspecific adsorption were evaluated by the injection of  $5.10^{-4}$  M of a FLU solution on the NIP-SAW sensor. Here also, no phase variations were recorded after the analyte injection.

On the basis of our recent work [11], quantum chemical calculations, based on the density functional theory (DFT), have been performed to understand the nature of the interactions involved between bipyrrole molecules (of the MIP matrix) and both FLU and Levo analytes. The bipyrrole molecules were optimized and placed in all possible interaction sites of each investigated analyte. The obtained complexes were fully optimized by DFT at B3LYP/6-311+G\* level [12-13] in both gas phase and in solution, by using the polarisable continuum model (PCM) and the Kohn Sham united atomic topologies cavities (UAKS) [14]. The analysis of vibrational frequencies indicated that optimized structures of complexes were at stationary points corresponding to local minima without imaginary frequencies. For each complex, the stabilization energy is computed as the difference between the energies of formed complex and isolated systems relaxed separately:

$$\Delta E_{T-X} = E_{T-X} - (E_T + E_X) \quad (1)$$

where T = bipyrrole and X = FLU or LEVO.

Fig. 2 shows the tridimensional structures of bipyrrole interacting with either flumequine or levofloxacin. The most favorable site is found to be the nearest carbonyl and alcohol groups. In both cases, the interactions between the dimer and the investigated analyte are due to the establishment of a strong hydrogen bond between the pyrrole amine and the carbonyl groups ( $\text{NH}\dots\text{O}=\text{C}$ ) of FLU or Levo combined to an  $\text{OH}-\pi$  formation.

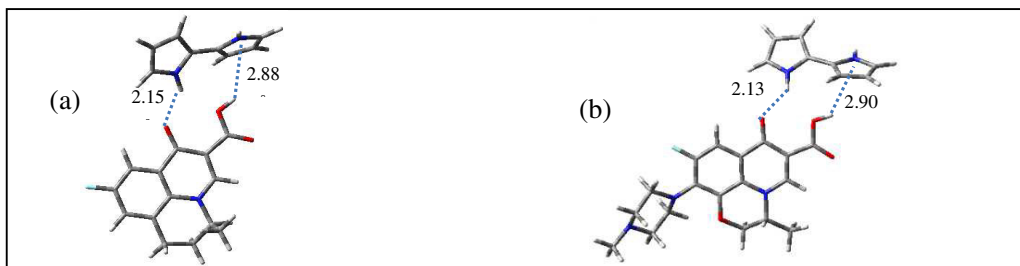


Fig. 2: Optimized structures of bipyrrole complexes with flumequine (a) and levofloxacin (b)

As MIPs are porous films, it is necessary to weight the computed interaction energies per molecular volume for each analyte. In water solvent we found - 69.7 and - 57.8 kcal/mol for flumequine and levofloxacin respectively.

The good agreement between gravimetric measurements and DFT calculations indicates that the detection of an analyte by a MIP is done through the establishment of chemical bonds between the matrix and the molecule on one hand, and by the perfect match between the molecule and the cavity on the other hand. Fitting the analyte molecule to the artificial receptor site is intimately related to a good orientation of the functions at the surface of the sensing polymer, which would be adapted to the shape and position of their counterparts in the target molecule.

## Conclusion

A sensitive SAW chemosensor functionalized with a thin layer of molecularly imprinted polypyrrole was designed for the detection of flumequine (FLU), the most used antibiotic for fish treatment in aquaculture ponds. The limit of detection was found to be about 1  $\mu$ M and the calculated sensitivity was of the order of  $9.4 \pm 0.4^\circ/\text{mM}$ .

The absence of phase shift after the injection of levofloxacin, is indicative of the high selectivity of the developed sensor toward FLU with respect to the fluoroquinolone group.

Quantum chemical calculations, based on the density functional theory (DFT) were in good agreement with experimental results. Moreover, weighting the computed interaction energies per molecular volume for each analyte, permits to take into account the porous morphology of a considered MIP.

This work highlights also the importance to combine theoretical and experimental results and demonstrates the need to take into account the surface coverage rate in studies involving molecularly imprinted polymers. From what has been stated above, electropolymerization of pyrrole on SAW transducers is a simple and efficient strategy for constructing gravimetric sensors for the direct detection of analytes in aqueous solutions, here antibiotics intensively used in aquaculture.

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## Note

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