

Love Wave Sensor Based on Thin Film Molecularly Imprinted Polymer: Study of VOCs Adsorption

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ABSTRACT

The overall objective of this work is to develop and to validate a quantitative, non-invasive diagnosis tool to monitor the efficiency of colorectal cancer chemotherapy. This paper deals with the validation of a molecularly imprinted polymer (MIP) thin film's coating process, allowing high sensitivity of the resulting microsensor. After description of the Love wave sensor and of the MIP coating process, the functionality of the resulting device is proved with electrical characterization. Deep characterization of the thin film - morphology and print effect - is proposed, based on dynamical responses observed under exposure to different vapors. Results point out a good reproducibility of polymeric films. MIP layers increased responses of sensors to vapors by a factor 3 to 4 compared to bare devices or to devices coated with non-imprinted polymer (NIP). For example, exposure to 4000mg/m³ (2126ppm) of ethanol in nitrogen induced a frequency shift of -1.4/-0.4kHz with MIP/NIP-coated sensor, respectively.

Index Terms: SH-SAW sensor, Polymer, MIP, Thin film, gas detection

I. INTRODUCTION

Colorectal cancer is the second leading cause of cancer related mortality. So it is very important to treat and detect colorectal cancer as early as possible because once it spread into lymph and other area of the body, it become harder to treat. The classic medical analysis tools such as therapeutic drug monitoring of cancer is limited and present inherent drawbacks such us : expensive, poor chemical and physical long term stability, skilled manpower, long time analysis [1].

Molecularly imprinted polymer (MIP) as recognition element of biosensors [2] [3] provide a promising alternative approach to overcome previous drawbacks. In recent years, molecular imprinting technology has found a broad range of applications in many areas such as chemistry, medicine, biochemistry or biotechnology. In this technique, functional monomers organized around a template molecule by non-covalent [4] or reversible covalent interactions [5], are enwrapped by the cross linker leading to the formation of a highly cross-linked network polymer.

At the end of polymerization and after removal of the template from the polymers, the resulting polymer exhibited binding sites specific to the template molecule [6] [7]. The synthesis technique is

simple, cheap and the obtained polymers present high selectivity, and excellent stability that promote their application in harsh environments, such as in organic solvent, or at high temperature, and made them ideal as recognition elements.[8] [9].

Mass-sensitive devices, such as the quartz crystal microbalance (QCM) or the surface acoustic wave component, are very advantageous for chemical sensing. In particular, Love wave devices have shown their great potentialities in several areas like biological liquid sensing [10] or gas detection [11], one of the highest that can be achieved with acoustic devices. This is mainly due to their guided nature in a thin layer, which ensures a high confinement of acoustic energy near the sensing surface. Recent studies showed many attempts to use acoustic wave sensor for biological detection of tumor markers with remarkable results. [12] [13].

In the present work, we developed a Love wave device based on a nucleotide (adenosine monophosphate: AMP) as model of target molecule for imprinted polymer [14]. The acoustic component and optimized coating process are described. Then, a characterization method based on real-time detection measurements of the coated sensor under ethanol and toluene exposure is proposed. Results are analyzed and lead to deeper understanding of the morphology of

the layer in terms of specific surface area in particular, by comparison with results obtained on reference devices (bare or coated with non imprinted polymeric film – NIP –). The printing effect is also validated by comparison of vapor detection measurements with MIP before extraction, after extraction and after recapture of the target molecule.

II. SH-SAW SENSOR

A. Transducing device

Figure 1 shows the basic configuration of the used Love wave sensor. It consists of delay lines built on an AT-cut quartz substrate. The Love wave is generated and detected by means of interdigitated electrodes (IDTs) deposited on the substrate and prior to SiO₂ guiding layer. They are composed of 44 splitted-finger pairs of gold and titanium (Ti/Au/Ti, total thickness about 150 nm) with a wavelength λ (spatial periodicity) equal to 40 μm . The acoustic aperture and the center to center distance of transmitting and receiving IDTs are equal to 40 λ and 210 λ , respectively. An orientation with a wave propagation direction perpendicular to the X-cristallographic axis is chosen in order to generate pure shear waves, allowing operation in a liquid medium. The SiO₂ layer is realized by plasma enhanced chemical vapor deposition (PECVD) to obtain a guided shear horizontal surface acoustic wave (guided SH-SAW) or Love wave. The acoustic energy is thus confined near the surface to maximize the sensor sensitivity. Detailed information about the delay-line technology can be found in [15].

B. Oscillator configuration

For experimental measurements, Love-wave delay lines were inserted in the feedback loop of a radiofrequency amplifier to compensate delay-lines losses and maintain oscillations.

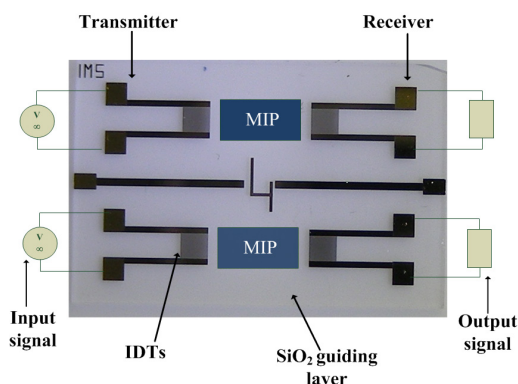


Figure 1. Love wave sensor (guided SH-SAW sensor).

C. MIP thin layer technology process

The critical point in coating acoustic devices is to obtain reproducible layers, with mechanical properties well adapted for wave propagation. The preparation of MIP thin film was based on the adaptation of a bulk polymerization technique which is often difficult to control or to reproduce. The principle adopted is based on spin coating method [16] [17] with control of deposition parameters.

Firstly, the SiO₂ surfaces of acoustic wave sensors were cleaned by soaking them for 15 min in piranha solution (H₂SO₄/H₂O₂) and immersed overnight in a solution containing 2% (v/v) of 3-(trimethoxysilyl) propyl methacrylate in toluene [18] in order to promote adhesion of the MIP layer.

Then, the pre-polymerization mixture containing the following components: functional monomer (acrylamide), template (adenosine monophosphate), porogenic solvent (dimethyl-sulfoxide) and initiator (2, 2-Azobisisobutyronitrile), was deposited by spin-coating at 2000 rpm for 10 s.

Finally, polymerization was initiated by UV light under inert atmosphere. For non-imprinted polymer, the procedure was similar except for the absence of print molecule (Fig. 2). Otherwise, the waveguide region was only covered with the MIP sensitive layer by masking IDTs with Kapton.

D. Characterization of the layer profile

The profiles and images of the MIP films were acquired using a stylus profilometer DektakÒ 150. The thicknesses of the resulting MIP and NIP thin films are in the order of a few hundred nanometers (between 200 nm and 250 nm), with edge effect due to the masking method used.

The film thickness is one of the main factors which affect the detection sensitivity and response time, it was adjusted in order to obtain an optimal value, also meeting the sensor insertion losses requirements.

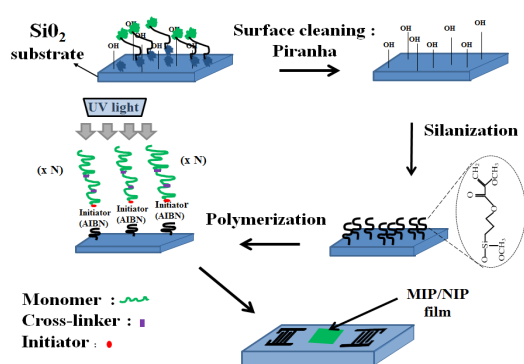


Figure 2. Schematic representation of the NIP/MIP coating technological process onto the acoustic wave sensor surface.

While the Scanning Electron Microscopy (SEM) in Fig 3, revealed the NIP and MIP films surfaces morphology and the pores sizes (range from 0.5 to 2 μ m).

E. Electrical characterization of coated sensor

The film coating changes the physical characteristics of the device. Indeed, due to the mass-loading induced by the MIP film, the wave is slowed down yielding an associated decrease in phase and in center frequency. Figure 4 shows the typical transmission characteristics S₂₁.

It can be noted on Figure 4.a that the deposition of the MIP layer induced no additional insertion losses. On Figure 4.b, the phase of the coated device appears slightly decreased corresponding to a decrease in frequency at constant phase as measured in oscillator configuration.

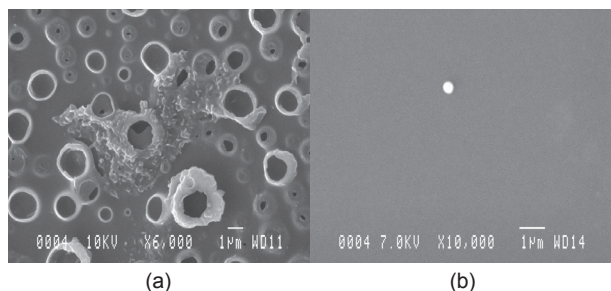


Figure 3. NIP/ MIP films pictures obtained using Scanning electron microscopy: (a) The NIP film morphology and (b) The MIP film showed a porous morphology with pores sizes ranging from 500nm to 2 μ m.

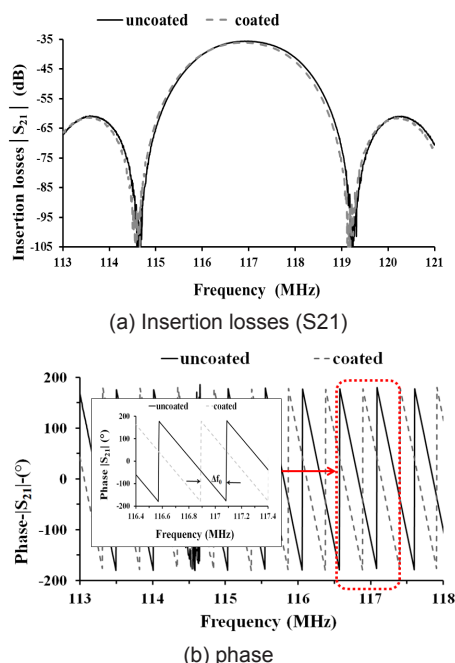


Figure 4. Transmission characteristics.

III. RESULTS AND DISCUSSION

Gas detection was realized with several vapors (toluene, ethanol) and concentrations flown past the sensitive part of the acoustic device placed in a hermetic chamber. Vapor at a constant flow rate of 0.1 l/min (dry air: nitrogen gas) has been generated using a gas generator (Calibrage, PUL 110, France). The ethanol concentration was in the range of 8-200 ppm. Regarding the toluene, concentration was in the range 8-1000 ppm. The sensor response was recorded in terms of frequency shift.

A. Morphology comparison between NIP and MIP thin films

The first purpose was to assess the morphology of the polymeric layers (porosity, specific surface area). The sensors used for these characterizations under gas exposure were subjected to the extraction step. Typical responses of the MIP- and NIP-coated Love wave device with each vapor are represented on Fig. 5 and Fig. 6. On each set of curves, typical responses of a bare device are represented as reference, with steady-state frequency shifts always close to -200/250 Hz, for both gases and regardless of the concentration. It can be observed a difference between dynamic responses to both gases, similar to previous observations [19].

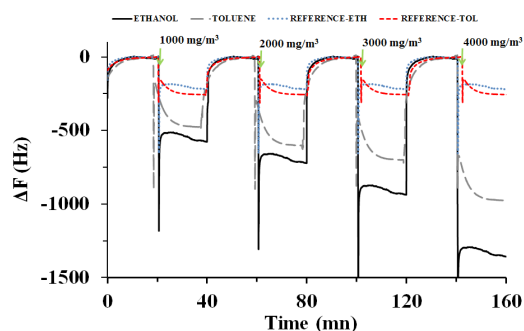


Figure 5. Real-time frequency of a Love wave sensor coated with a 200 nm MIP layer in response to toluene and ethanol concentrations steps.

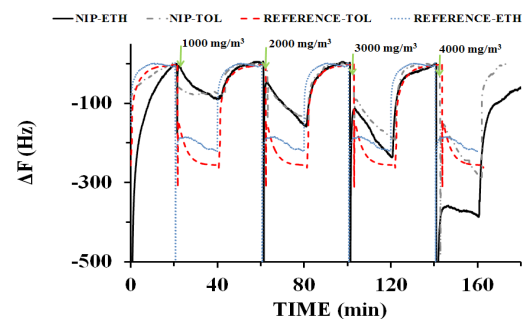


Figure 6. Real-time frequency of a Love wave sensor coated with a 200 nm NIP layer in response to toluene and ethanol concentrations steps.

Indeed the sorption kinetics is slightly faster for ethanol than toluene, on the reference device, as well as on the coated devices. This demonstrates a significant difference between the interactions of the two gases with the MIP sensitive layer. This could be used as a tool for discrimination of the two vapors even at identical concentrations. In addition, the frequency reaches a steady-state after few minutes for toluene and less than 1 minute for ethanol.

A higher sensitivity is clearly observed with the MIP layer rather than the NIP layer, with a factor 3 to 4. Indeed, the MIP possesses a high pore volume and specific surface area, thanks to the presence of specific recognition sites in the polymer which are complementary to the template molecule both in shape and in arrangement of functional groups.

B. Imprint effect

The other aim was to evaluate the imprint effect by realizing gas detection before removal of template from the MIP film, after removal and then after rebinding of this AMP molecule. The real-time responses to ethanol and toluene exposure are represented in Fig. 7 and 8. The results point out curve shapes similar for the three investigated steps, with a change in frequency dependent on the gas concentration. Above all, it was also observed that the frequency shifts of the coated

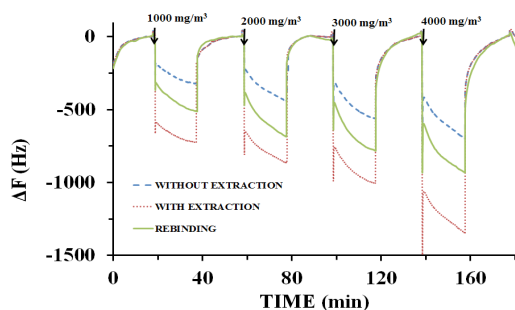


Figure 7. Illustration of formation and breaking of the binding template (AMP) /monomer with a film coated Love wave device to toluene exposure.

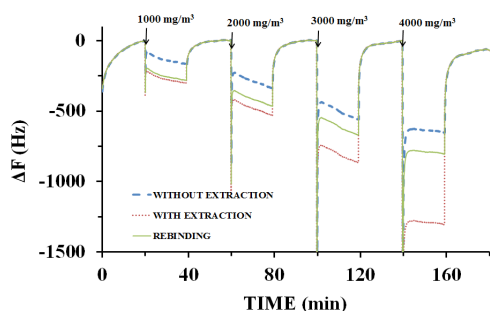


Figure 8. Illustration of formation and breaking of the binding template (AMP) /monomer with a film coated Love wave device to ethanol exposure.

sensors, with identical vapor and concentration values, obtained before removal, after removal and after rebinding steps, were different and in good agreement with expected behaviors.

Before removal of the template, all recognition sites were occupied by AMP molecules, so the specific surface area is the smallest. After removal of the template from the polymer matrix, most recognition sites became available, so the specific surface area of the MIP film increased and thus the sensitivity. After interaction with AMP, some of these recognition sites were occupied again, inducing a decrease of the specific surface area. These results are consistent if we consider a mass effect, due to the presence of gas molecules into the empty cavities.

IV. CONCLUSION

A structure combining the advantages of the high selectivity of MIP and high sensitivity of Love wave sensors was proposed. The molecular thin films have been deposited directly onto the sensor surface by spin coating in nanometer range. In the experimental setup, the response of MIP coated sensor under several gas concentrations was investigated. The results show that the sensitivity of the NIP-coated sensor was much smaller than that obtained for the MIP-coated sensor, with a factor 3 to 4. For comparison, the frequency variation of the bare device was low and roughly the same regardless of the concentration of ethanol or toluene. Furthermore, the imprint effect was assessed by realizing gas detection with MIP- and NIP- coated sensors after the extraction of AMP from the MIP film and after rebinding of the AMP molecule. For example, exposure to 4000mg/m³ (1063 ppm) of toluene induced a frequency shift of -0.5 kHz before extraction, -1.4 kHz and -0.9 kHz respectively after extraction and rebinding steps.

The proposed characterization technique thus enables to evaluate qualitatively the porosity of MIP and NIP thin films, and gives information on surface morphology of thin MIP films. Further work will focus on realizing nucleoside (colorectal cancer biomarkers) detection with a Love wave device coated with a new MIP being synthesized, and combined with a microfluidic system.

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