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Love Mode Surface Acoustic Wave and High Fundamental Frequency Quartz Crystal Microbalance immunosensors for the detection of carbaryl pesticide

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Abstract

In this work we determined the Sensitivity (estimated as the I_{50} value) and Limit of Detection (LOD) for the immunodetection of carbaryl pesticide with two different types of acoustic wave sensors: High Fundamental Frequency Quartz Crystal Microbalance (HFF-QCM) and Love Mode Surface Acoustic Wave (LM-SAW). Results were compared with others previously reported using different sensors and techniques, like traditional QCM, Surface Plasmon Resonance (SPR) and Enzyme-Linked ImmunoSorbent Assay (ELISA). We used the AWS-A10 research platform (AWSensors, Spain) to perform the experiments. We obtained I_{50} values of 0.31 $\mu\text{g/L}$ and 0.66 $\mu\text{g/L}$, and LODs of 0.09 $\mu\text{g/L}$ and 0.14 $\mu\text{g/L}$, for 120 MHz LM-SAW and 100 MHz HFF-QCM devices, respectively. Both the sensitivities and LODs of the immunosensors improved previously reported SPR and QCM results by one and two orders of magnitude, respectively, and reached those of ELISA.

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1. Introduction

Acoustic wave sensors are widely used in in-liquid biosensing applications [1]. One of these applications is the label-free pesticide analysis in fruit and juices [2]. However, the sensitivity and LOD of acoustic immunosensors should be improved to comply with regulations and to extend their applicability to more demanding applications, such as the analysis of drinking water. In this work we propose two acoustic technologies, HFF-QCM and LM-SAW, for the detection of a Low Molecular Weight (LMW) compound, carbaryl pesticide. Carbaryl was chosen as model analyte since it was used as a reference LMW compound by previous works, both for acoustic and other techniques such as SPR and ELISA [2,3,4]. We sought to enhance the analytical performance of the proposed acoustic biosensors by improving the sensitivity of the transducer devices -using sensors working at higher frequencies than those previously used in this application -, and by reducing the readout system noise and improving the flow injection analysis (FIA) system - using a dedicated system to perform the experiments -.

2. Materials and methods

2.1. LM-SAW sensors, HFF-QCM sensors and flow cells

The Love-Mode sensors used were based on a 17×8.4 mm and 0.35 mm thick single-side polished Z-propagating AT-cut quartz substrates. The silicon dioxide (SiO₂) guiding layer had a thickness of 3 μm. The input and output IDT consisted of 100 double-finger pairs designed for a sensor synchronous frequency of 120 MHz. The sensing area consisted of 10 nm of chrome and 50 nm of gold.

The HFF-QCM sensors used were based on AT-cut 100 MHz HFF-QCM inverted mesa resonators. The electrode had 1mm of diameter and its structure consisted of a gold layer of 90 nm deposited over a 5 nm chromium adhesion layer. The resonators were bonded permanently to a support made of polyether ether ketone (PEEK) using an epoxy adhesive. The electrical contact between resonator electrodes and copper pads in the lower part of the peek support was made by a conductive epoxy.

Flow cells for both types of sensors were designed and manufactured considering the mechanical, electrical and chemical requirements of the application. They allowed a fast and easy installation and replacement of the sensors, and a convenient interface to the system (Figure 1b).

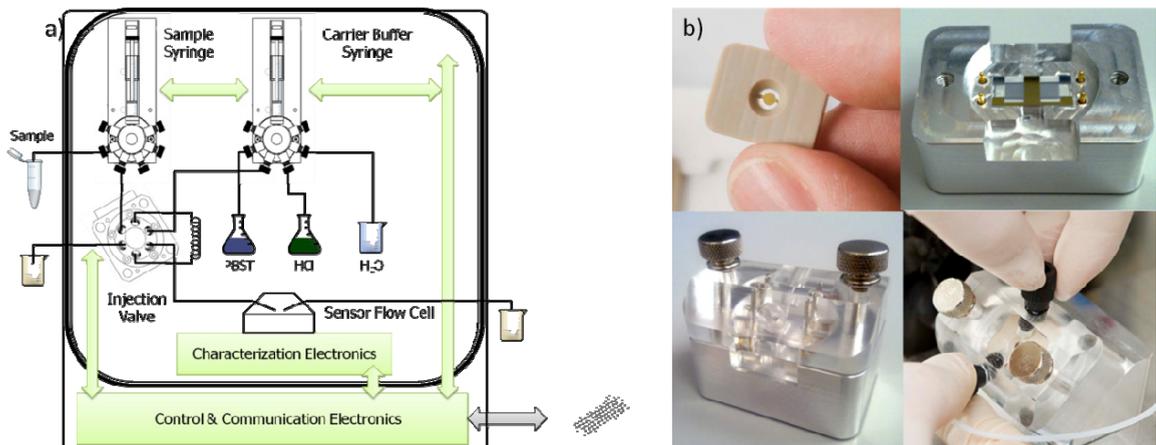


Fig. 1. (a) Block diagram of the AWS A10RP system; (b) Picture of the sensors and flow cells used in this work.

2.2. Experimental set-up

We used the research platform AWS-A10 (Advanced Wave Sensors S.L., Spain) to perform the experiments. The platform consists of an electronic characterization system, an automated FIA system, and a control and communications system (Figure 1a). The proposed system allows the comparison of both devices, measured by the same characterization system and under similar experimental conditions. The electronic characterization system is based on the open loop phase detection technique at a fixed frequency, which allows the characterization of very high frequency acoustic sensors without a significant increase of noise [5,6,7]. A PC software collects data from the characterization system and controls all the elements of the AWS-A10 Platform, including the FIA system. All measurements were performed at a temperature of $25^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$.

2.3. Functionalization protocol

The sensing areas of the sensors were functionalized following a similar procedure, chemicals and reagents as described [2]. Sensors were cleaned by exposure to UV/ ozone using the UV/Ozone ProCleaner (BioForce Nanosciences Inc., USA). After exposure, sensors were rinsed with distilled water and ethanol, and dried with nitrogen gas. Active areas were functionalized by immobilizing BSA-CNH carbaryl hapten conjugates ($10\mu\text{g/mL}$) through the formation of a mercaptohexadecanoic acid self-assembled monolayer (SAM).

3. Results

The immunoassays were competitive inhibition tests based on the conjugate coated format. A fixed concentration of $2\mu\text{g/mL}$ of the monoclonal antibody (Mab) LIB-CNH45 [3] was mixed with standard solutions of the analyte at different concentrations. In this format the analyte inhibits the antibody binding to the immobilized conjugate. Thus, increasing concentrations of analyte reduce the measured signal changes.

Figure 2a shows the immunoassay cycle followed for each sample. The protocol consisted of pumping phosphate buffer solution containing 0.005% Tween (PBST) at a flow rate of $20\mu\text{L/min}$ at least for 5 min (only 1 min. shown in figure), next injecting the sample ($250\mu\text{L}$) into the main flow for 15 min at the same flow rate. At this point, the change in the phase signal with respect to that obtained at the time of sample injection was measured ($\Delta u_{\phi} = u_{\phi} - u_{\phi 0}$). The regeneration of the active area was performed by pumping 0.1M HCl for 4 min and PBST again for 4min., at a flow rate of $250\mu\text{L/min}$.

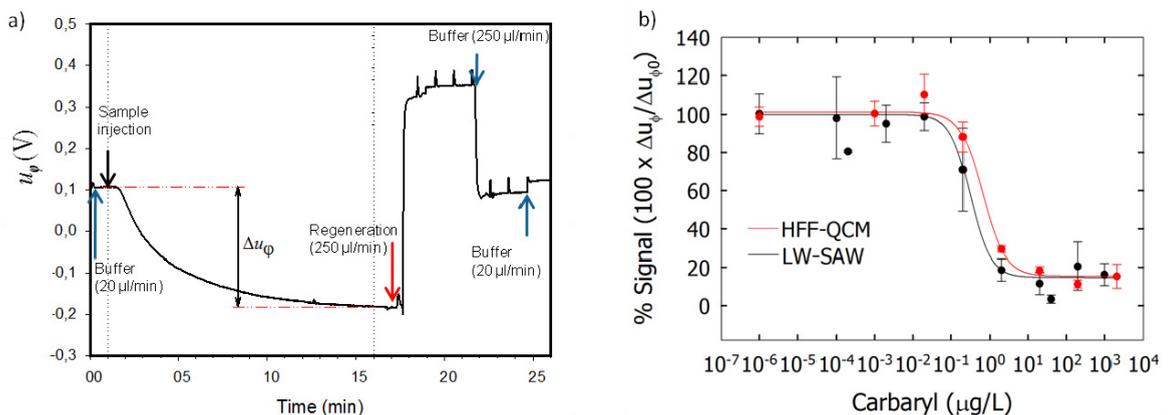


Fig. 2. (a) Immunoassay cycle; (b) LM-SAW and HFF-QCM standard calibration curves for carbaryl determination

All samples were injected and measured at least in duplicate. Figure 2b shows the standard curves obtained by representing the phase signal change versus the analyte concentration. The measurements were normalized as the

percentage of the maximum signal (the phase signal change in the absence of analyte). Experimental points were fitted to a four-parameter logistic equation and the analytical parameters were extracted from the fitted curve.

The developed immunosensors achieved I_{50} values of 0.31 $\mu\text{g/L}$ and 0.66 $\mu\text{g/L}$, and LODs of 0.09 $\mu\text{g/L}$ and 0.14 $\mu\text{g/L}$, for 120 MHz LM-SAW and 100 MHz HFF-QCM devices, respectively (Table 1). We compared these results with previously reported ones, using different technologies but the same target analyte and immunoreagents. The sensitivities and LODs of the developed immunosensors improved those of SPR [4] and QCM (with classical frequency shift measurement [2] and the phase shift measurement approach [8]). The improvement was in the range of one and two orders of magnitude respectively. Analytical parameters also approached ELISA's ones [3].

Table 1. Analytical performance of carbaryl determination for different immunosensing techniques

	ELISA [3]	SPR [4]	QCM 10MHz Δf [2]	QCM 10MHz $\Delta\phi$ [8]	HFF-QCM 100MHZ	LM-SAW 120MHz
Sensitivity ($\mu\text{g/L}$)	0.72	3.12	30.00	16.70	0.66	0.31
L.O.D ($\mu\text{g/L}$)	0.13	1.41	11.00	4.00	0.14	0.09
Working Range ($\mu\text{g/L}$)	0.23 - 2.36	1.90 - 5.75	15.00 – 53.00	7.00 – 35.00	0.26 – 1.73	0.14 – 1.63

4. Conclusions

We have developed both HFF-QCM and LM-SAW immunosensors for the detection of a LMW compound, the Carbaryl pesticide. The enhancement of the analytical performance sought was accomplished. Compared with QCM results previously reported, we achieved an improvement of about two orders of magnitude in sensitivity and LOD with both technologies. The obtained I_{50} and LOD values also exceed by one order of magnitude those reported for SPR and reached those of ELISA. The high sensitivity reached by these immunosensors could allow label free pesticide analysis at concentrations close to the European Maximum Residue Levels for drinking water.

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