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Direct comparison of the sensitivity of QCMs and AlN-based TFRs biosensors

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Abstract

We present the direct comparison of the performance of two gravimetric biosensors based on acoustic resonators, a quartz crystal microbalance and a high frequency AlN-based bulk acoustic wave film solidly mounted resonator (SMR). Both sensors are functionalized with streptavidin to detect the response to TBA29 aptamer biotin modified and different concentrations of thrombin. Experimental results reveal that both sensors succeed in detecting the targeted species, although SMRs show significantly greater sensitivity and a lower limit of detection.

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Keywords: Biosensor, quartz crystal microbalance; thin film resonators; AlN

1. Introduction

Biosensors are the most promising tools in identification of biomolecules, bacteria, virus or toxins from clinical diagnostics to food analysis, bioprocess and environmental monitoring. Biosensors are essential tools for early bio-recognition of pathogens in different areas, such as health, food industry or environmental protection. They offer high specificity and sensitivity, allowing the detection of a broad spectrum of analytes in complex sample matrices (blood, serum, urine, food, soils, waste water, etc.), with minimum sample pretreatment. Contrary to complex laboratory techniques, like ELISA, real time PCR, or HPLC, that require specialized equipment and trained users, biosensors offers the prospect of a simple, highly specific, fast, cheap, and label-free sensing procedure for massive analysis[1].

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Gravimetric biosensors are specific devices that may meet all these features. They detect the mass variation of a device in response to the specific binding of an antigen to their surface. Piezoelectric thickness-mode resonators are common gravimetric sensors that respond to mass changes with a shift of their resonant frequency. Among them, the most typical is the quartz crystal microbalance (QCM) formed by a quartz single crystal plate sandwiched between a couple of thin film metallic electrodes that can be functionalized with receptors (antibodies or aptamers) to a specific antigen [2,3].

According to Sauerbrey's formula [4], which states that the sensitivity of a resonator increases with frequency, greater sensitivities could be achieved in thickness-mode resonators by reducing the thickness of the piezoelectric layer. However, since quartz plates can only be thinned to a limited value, thin film resonators (TFR) operating in the GHz range based on AlN and ZnO films are being developed to improve the performance of gravimetric biosensors. As is the case in AT-cut QCMs, to reduce the radiation of acoustic energy to liquid media shear-mode TFRs containing wurtzite layers with tilted microcrystals operating in shear mode are preferred to TFRs operating in longitudinal mode [5,6]. One of the limiting factor in this kind of sensors is the accuracy in the determination of the resonant frequency shift. The inherent noise of the devices and the electronics used for detecting the frequency are the responsible of fixing the noise floor, which limit the resolution of the measurement and the limit of detection (LOD).

In this work, we carry out a direct comparison of the performance as gravimetric biosensors of QCMs (AT-cut) operating at 5 MHz (fundamental frequency) and AlN-based TFRs operating in the shear mode at 1.4 GHz, in order to verify whether the significant increase of the resonant frequency may lead to better functional properties of the biosensor. Both sensors are fabricated by using materials and techniques as similar as possible, in order to minimize the influence of issues different from the operation frequency in the performance of the devices. We pay a special attention in minimizing the effect of ambient magnitudes, such as changes in temperature or liquid viscosity or density, adherence of other undesired species (nonspecific binding), changes in the pressure inside the liquid, etc. in order of obtaining the LOD only due to the intrinsic noise.

2. Materials and Methods

QCM were achieved by depositing Cr/Ir or Cr/Au electrodes through a mechanical mask over the two sides of standard AT cut quartz crystals 14 mm in diameter. The contacts defined an active area of 0.78 cm^2 (a circle of 5 mm in diameter). TFRs consisted in a piezoelectric AlN stack formed by an AlN film with grains tilted 25º with respect to the normal sandwiched between two iridium electrodes, which allowed the effective excitation of both shear and longitudinal modes. The AlN active layers were sputtered through a two-stage sputtering process [7]. The Ir tracks to the connection pads were regrown with a 2 µm-thick Mo layer to reduce the series resistance of the resonator. The acoustic isolation of the piezoelectric stack was achieved by using a fully insulating acoustic reflector that alternated SiO_2 and Ta_2O_5 films of low and high acoustic impedance, respectively.

The top electrode of both TFR and QCM was covered with a 50 nm-thick sputtered SiO_2 film that was functionalized using a standard APTES ((3-Aminopropyl) triethoxysilane)-GA (glutaraldehyde) functionalization protocol [8]. The ultimate aim of the functionalization process was to cover the entire active area of the resonator with receptors tightly bound to the surface with the right orientation, so that they could expose their active side to the targeted species. To achieve this goal we took advantage of the high affinity of streptavidin to biotin, and the fact that any receptor can be biotin-modified at any position within the molecule. The reagents used were APTES 2% in ethanol, glutaraldehyde (GA), streptavidin 10 µg/ml in NaCl 50 mM, thrombin-binding aptamer (TBA29) (all from Sigma-Aldrich).

QCMs and TFRs were characterised by measuring their electrical impedance around the resonant frequency with an Agilent N5230A network analyser. TFRs were contacted using calibrated RF probes. A specific fluidic system made of a laser-cut methacrylate sealed with a nitrile O-ring was used for in-liquid measurements in both cases. An injection system and a micro-valve setup enabled feeding the liquid inside the sensing chamber of 30 µl of capacity

at rates from 10 µl/min to 500 µl/min. The accurate tracking of the resonant frequency was achieved by fitting the maximum of the real part of the admittance to an eight degree polynomial in a narrow frequency interval; the roots of its first derivative were calculated and the resonant frequency identified. This procedure was implemented in a LabView® application that enabled measuring the frequency with 2 kHz accuracy each 3 seconds for TFRs, and 1.5 Hz each 7 seconds for four harmonics of the QCM. In the end, the signal from the third harmonic at around 15 MHz was chosen as the most representative.

3. **Results and discussion**

First, we assessed the frequency response of the two sensors both in air and in liquid (binding buffer tween-20 0.05% BSA 0.1% (BB-T BSA)). We observed that during in-liquid operation, the quality factor decreased, from 115000 to 3500 in QCMs and from 250 to 190 in TFRs, owing to the influence of the density and viscosity of the solution. Since these magnitudes could mask the gravimetric effect, the buffer solution was first circulated over the sensors before feeding the solution containing the targeted species to the reaction chamber. The very low concentrations of TBA29 and thrombin used do not appreciably change these magnitudes.

The active area of both sensors was covered with streptavidin as upper layer of functionalization. The functionalization protocol was carried out by using rigorously the same protocol for both sensors as described above. To compare the performance of both sensors we use two kind of molecules with different molecular weight and affinities. The biotin modified TBA29 aptamer (9.5 kDa) to thrombin was bound to streptavidin. TBA29 will work as receptor for human thrombin (36 kDa) which is the second added molecule. First, we injected TBA29 aptamer at 640 nM in BB-T BSA, while monitoring the frequency variation with time, followed by different concentrations of human thrombin.

In Fig. 1, the frequency variations with time is shown for both sensors. It is worth to note that the total frequency variation for TBA29 is 2.5 ppm for the QCM (Fig 1(a)) and around 80 ppm for the SMR (Fig 1(b)). Noise levels are around 0.1 ppm for QCM and around 2 ppm for the SMR, all in these particular devices, which are representative of the typical measurements made with both sensors. It is also interesting to highlight two different magnitudes in the measurement: total frequency shift and the slope of the curve. The first magnitude is related with the mass sensitivity of each sensor. The slope of the curve depends on reaction kinetics including the mechanism of reactive arrivals to the surface. The affinity reaction in the first step was between biotin and streptavidin with K_d = 4 x 10^{-14} M. In the second step for TBA29 and thrombin K_d = 10^{-10} M.

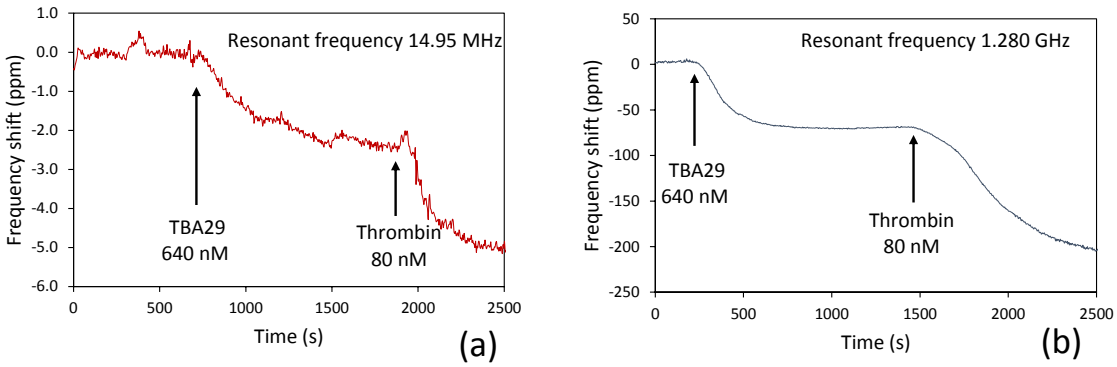

Fig. 1. Time evolution of the frequency shift in a detection process of TBA29 (640 nM) followed by a thrombin (80 nM) for (a) a QCM working in the 3th harmonic and (B) an SMR working in fundamental mode.

There are important differences between the size and form of each chamber in both sensors that are imposed by the sensor geometry (60000 μm^2 in SMR and 19.6 mm^2 for QCM). Diffusion of species to the surface is the main mechanism of limiting the reactions so velocity of reaction will be slower in QCM as volumes are larger.

Noise levels are around 0.1 ppm for QCM and around 2 ppm for thee SMRs all in these particular devices, which are representative of the typical measurements made with both sensors. We have repeated this detection process for different concentrations of thrombin from 4 nM to 80 nM in order to find the LOD of each sensor. In figure 2 the results of these experiments are shown. Apart of the differences in the ranges of operation of the two sensors, it is worth to note that a concentration of 4 nM thrombin is under the LOD of the QCM.

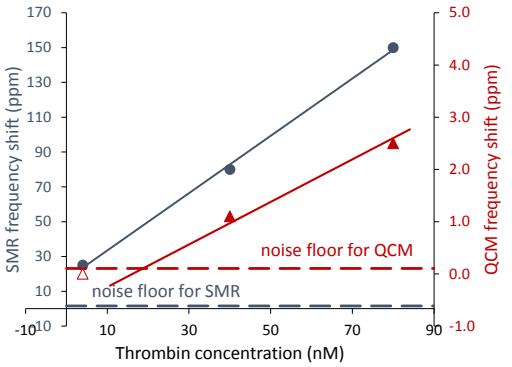

Fig. 2. Maximum shift achieved for the frequency shift of both types of sensors vs. the thrombin concentration. Noise floor for each sensor is also shown

As shown in these experiments the SMR sensors show a better performance than QCM ones when are used in the same conditions. We think that the LOD of the SMR is below the 4nM concentration used in this work. Aspects as the fluidic system could be improved to allow a better kinetics of the reaction, particularly in SMR case.

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