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ScienceDirect
Sensors and Actuators B 127 (2007) 248–252
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Immunosensor utilizing a shear mode thin film bulk acoustic sensor

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Available online 17 July 2007

Abstract

An AlN thin film electro-acoustic resonator has been fabricated employing a reactive sputtering process for the deposition of an AlN thin film with inclined *c*-axis for excitation of the shear mode for operation in liquid media. The main objective is to investigate the efficiency of the micro-fluidic channel system integrated in the silicon wafer underneath the AlN resonator. A comparative study between the shear mode thin film bulk acoustic resonator (FBAR) and a quartz crystal microbalance (QCM) using a competitive antibody–antigen association process for detection of drug molecules is presented.

Keywords: Competitive immunosensor; Shear mode FBAR; Drug detection

1. Introduction

In recent years there has been substantial progress in the thin film electro-acoustic (TEA) technology for the fabrication of thin film bulk acoustic resonators (FBAR) utilizing the thickness excited shear mode for sensing in liquid media [1,2]. Reactive sputtering is the preferred method for depositing the piezoelectric material (AlN, ZnO, etc.) owing to its numerous advantages not the least being part of the planar technology. This opens the possibility for mass production of integrated sensors. The TEA technology has also the advantage, compared to the single crystal case, that low cost devices operating in the GHz range can now be fabricated, implying the fabrication of low cost, miniature biosensors with extreme sensitivities.

There exists certain skepticism as to whether the FBAR sensor technology would have a higher mass resolution as compared to quartz crystal microbalance (QCM) since the $f \times Q$ product of the FBAR materials is much lower than that of quartz. Recent noise studies [3,4] of FBARs performed with a Network Analyzer, however, indicate that the mass resolution of AlN FBARs operated in liquids is equal to if not better than that of QCM, despite the fact that the FBARs were evaluated under non-controlled conditions. Admittedly, the mass resolution was estimated under the assumption that the sensitivity obeys the Sauerbrey equation.

In a further development, to improve their temperature stability a practically complete temperature compensation (in the range 25–95 °C), of a shear mode AlN FBAR, has recently been demonstrated by employing a SiO_2 compensation layer [5]. This is believed to further improve the stability of the FBAR for future applications.

Returning to the discussion on mass resolution it has also been shown [4] that FBARs must be viewed as heavily loaded resonators, since the non-piezoelectric electrode metal layers make up a considerable part of the resonator acoustic path. This results in a non-linear behavior of the mass sensitivity with mass loading and more specifically loaded resonators exhibit a substantially increased mass sensitivity. Thus, in the same study [4] it is shown that the mass sensitivity of an FBAR resonator additionally loaded with an Au layer (needed for chemical stability) increases the mass sensitivity by a factor of two as compared to that calculated from the Sauerbrey equation.

All these findings strongly indicate that FBAR-based biosensors would exhibit both high sensitivity and high resolution. Nevertheless, there are still a number of issues which need to be addressed, namely definition of the micro-fluidic system and choice of materials, wetting of the sensors surface, sensor functionalisation and packaging, the influence of the high frequency on the biochemistry, integration with the read-out electronics, etc.

The focus of this work is to experimentally study the mass transport efficiency of the integrated micro-fluidic transport system of the FBAR by performing a comparative study between a shear FBAR operating in the GHz range and a QCM in the MHz

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doi:10.1016/j.snb.2007.07.051

frequency range. To make a meaningful comparison, however, a number of issues had to be considered in order to account for the inherent differences between the QCM and the FBAR sensor. First the higher frequency of operation result in a shorter sensing length of the FBAR to be shorter than for the QCM due to shorter penetration depth of the acoustic shear wave in liquid media. To investigate the sensing length of the FBAR sensor is out of the scope of this study. It is, therefore, most practical to use a chemistry where the sensing occurs close to the surface. In addition, the chemistry must be well established and robust so that differences in surface roughness and quality could be easily eliminated. The reaction should be of high affinity but have a short critical distance for reaction so that reaction only occurs if the species are brought together in close proximity by flow and/or diffusion.

In view of this a commercial affinity-based chemistry developed by Biosensor Applications AB, which utilizes a competitive antibody–antigen association process for the detection of drug molecules in a rough environment was chosen. In the case of small molecules such as narcotic analytes the competitive binding will also give higher sensitivity, as described below. Two types of drug analytes, cocaine and heroin respectively, have been tested together with their corresponding biochemistries.

2. Theory

The interaction between an antibody and its antigen depends both on the adsorption and desorption kinetics of the system as well as on their transport to the surface. The response of the sensor will therefore depend on both the reaction kinetics and the mass transport of the reactants. The slowest of these factors will set the limitations on the reaction rate and consequently on the time response of the sensor. Thus, detection of small amounts of target molecules with a comparably large number of specific detection sites on the sensor surface a reaction will take place as soon as the reactants come into contact, hence making the system predominantly mass transport limited. The rate of forming an analyte–antibody complex can be described with the mass transport model:

$$\frac{d[AB]}{dt} = k_m([A_0] - [A_s]) \quad (1)$$

where $[AB]$ the concentration of the analyte–antibody complex, k_m the mass transport coefficient, $[A_0]$ the analyte concentration in bulk of the sample solution and $[A_s]$ is the analyte concentration within the critical distance where reaction with a ligand is possible. The area near the ligand will be basically depleted of free analytes and $[A_s]$ is zero, if the system is true mass transport limited. A sufficient flow near the sensor surface will therefore be of considerable importance.

3. Experimental

3.1. Resonator fabrication

Recently a two-step reactive sputtering process for depositing AlN with 30° inclined *c*-axis has been developed [2]. This has enabled deposition of films that can be operated in the thickness excited quasi-shear mode, with electro-mechanical coupling (k_t^2) of around 2%, which is suitable for operation in liquid media. The same deposition process is used here for depositing a tilted AlN film between two aluminum electrodes onto a supporting Si-wafer. The silicon is etched by a standard BOSCH process from the backside defining thus the free standing membrane (see Fig. 1). This is done both to isolate the resonator acoustically from the substrate and to create a cavity with a volume of about 0.1 μL underneath the resonator for the liquid to be analyzed, making the grounded bottom electrode the sensing surface of the resonator. The cavity is further connected to the top of the Si surface through a series of horizontal and vertical channels, which form the micro-fluidic transport system for analyte delivery to the bottom electrode of the resonator, see Figs. 1 and 2. Since the width of the horizontal channels is intentionally made smaller than the width of both the vertical channels as well as the cavity then owing to the so-called aspect ratio dependent etching the Si etch rate in the horizontal channels will be the lowest. This results in that the Si wafer is not fully etched in these areas which naturally defines a bridge underneath the AlN film outside the cavity for mechanical stability (see Figs. 3 and 4) as the micro-fluidic system is to be subsequently connected on wafer with the input and output analyte reservoirs through an O-ring seal. As seen in Figs. 3 and 4 the Si bridge has a thickness of about 30–50 μm with a very smooth and broad transition region while the channels as a whole is void of sharp edges.

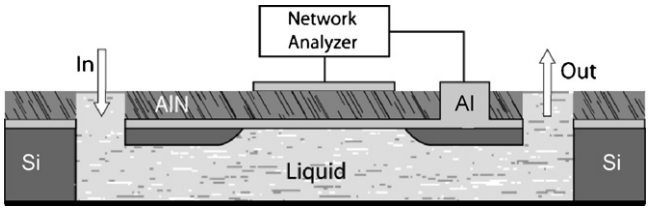

Fig. 1. Schematic illustration of a shear mode FBAR resonator together with a micro-fluidic transport system.

Next, the backside of the wafer is deposited with a 10 nm titanium adhesion layer followed by a 20 nm thick Au film by thermal evaporation providing thus a suitable surface for

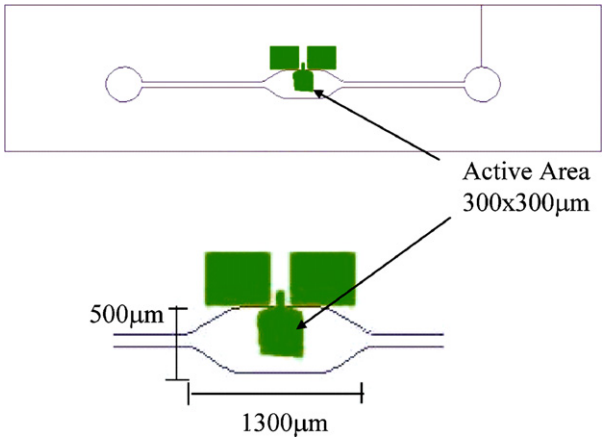

Fig. 2. Schematic illustration of the micro-fluidic transport system (top view) and the location of the active area.

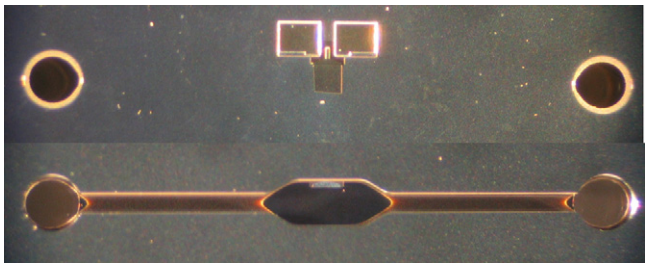

Fig. 3. Photographic images of the active area seen from above together with the circular channel openings on each side and the channel system seen from underneath respectively.

the subsequent biochemical reactions. Finally, the micro-fluidic transport system is enclosed from the backside with a plastic tape. The active area of the sensor is 0.09 mm^2.

3.2. Competitive binding

Affinity-based sensors can be operated based on detection with direct, competitive or displacement binding; where direct binding is by far the most commonly used. In some cases it may however be of interest to consider other detection systems, since higher sensitivity can be obtained. In the case of gravimetric sensing such as FBAR and QCM the sensing is based on a frequency shift caused by mass changes on the surface. Therefore, the actual mass of the species bonding to or leaving the surface is of significant importance. In the case of cocaine and heroin the mass of the analyte molecules is considerably smaller than the mass of the antibodies, implying that a release of the antibody would cause a larger frequency shift as compared to the opposite reaction. In other words, this approach provides natural mass amplification and hence increases the sensitivity of the sensor, particularly for low concentrations of the target molecules. In other words, this approach effectively improves the lower detection limit. In addition, such a competitive binding would result in a slight increase in Q of the resonator (and hence resolution) due to a corresponding decrease in the viscous load caused by the large antibody molecules.

Surface functionalization for competitive binding is done as follows. First, a synthetic antigen is attached to the Au surface of the resonator described above. In the second step antibodies with specific affinities towards both the antigen and the target molecules are attached to the antigen (see Fig. 4A). More specifically, the affinity of the antibodies to the target molecules is stronger than that of the antigen. At this stage the bottom surface of the resonator is functionalized and the biosensor is ready for use. Finally, introducing an analyte containing the target molecules would result in a reaction between the latter and the antibodies. The reaction complex is subsequently released from the surface due to weakened binding (see Fig. 4B) and will be detected by the resonator as a negative mass load.

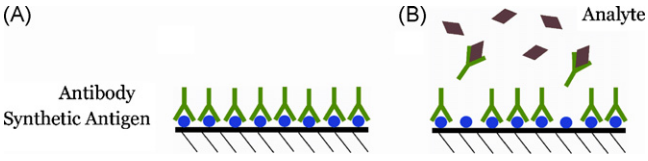

Fig. 4. The antibodies bind to a synthetic antigen onto the surface of the resonator (A). The antibodies bind with higher affinity to the analyte and will therefore leave the surface (B).

In view of this study the main benefit from using this competitive binding is that the sensing occurs close to the surface eliminating the expected difference in decay length of the QCM and FBAR.

3.3. Measurement setup

The QCM measurements were carried out with a commercial instrument from Biosensor Applications AB for detection of drugs or explosives [6]. The FBAR sensor was characterized with a HP 8720D Network Analyzer in a one-port configuration. The series and parallel resonance frequencies were extracted from the maximum of the real part of the admittance and the impedance respectively. No specific precautions have been taken to control the temperature, exposure to light, vibrations, that is, the measurements were performed in an ordinary office environment.

The same proprietary biochemistry provided by Biosensor Applications AB was used in both cases. The two measurement set-ups correspondingly adapted and modified to provide as equivalent conditions as possible. The flow rate was kept at 100 μL/min. The enclosed channel system of the FBAR sensor makes it difficult to directly access to the sensing surface for which reason the functionalization of the surface was made by flowing the chemicals through the micro-fluidic system.

4. Results and discussion

Measurements were performed using the competitive binding protocol described above for the detection of cocaine and heroine. Specifically, the surface is initially saturated with an antigen and the antibodies are then injected and adsorbed onto the surface. When the analyte is injected, antibodies are released from the surface and bind to the analyte in the solution instead, due to the higher affinity. Hence, the final positive frequency shift is a measure of the concentration of the cocaine analyte. Fig. 5 represents a sequence of measurements with the FBAR sensor

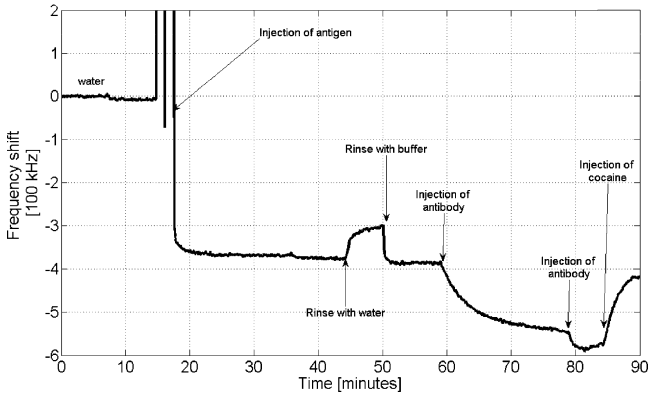

Fig. 5. Frequency shift vs. time illustrating surface functionalization and detection of cocaine with competitive binding.

Table 1
Frequency shifts of 10 MHz QCM and 800 MHz FBAR biosensor

	QCM (Hz)	Estimated for FBAR (kHz)	Measured for FBAR (kHz)
Cocaine (2.5 ng)	15–25	43–72	27
Heroin (2.5 ng)	10–15	29–43	42

Estimation of expected frequency shifts for the FBAR according to the Sauerbrey equation are also given.

illustrating all steps involved, namely from surface functionlization through to cocaine detection. In this case the concentration of cocaine (1 ng/μL) is so high that almost the whole sensor surface is depleted from the antibodies attached onto the latter during the functionalization step. To establish a mass transport limited regime required for the characterization of the micro-fluidic transport system, however, much smaller concentrations and amounts of the analyte are to be used. Further, the mass sensitivity is used as an indirect measure of the effectivity of the micro-fluidic system since the transport of the analyte to the sensor surface will directly influence the mass sensitivity in the transport limited regime. Specifically, the mass sensitivities of both 10 MHz QCM and 800 MHz FBAR biosensors towards 50 μL cocaine and heroin analytes with a concentration of 50 pg/μL each (i.e. total amount of 2.5 ng each) were then measured. In addition, the mass sensitivities were calculated using the Sauerbrey equation. The results are presented in Table 1.

As indicated in the introduction above several studies have recently shown that the Sauerbrey equation actually was found to underestimate the mass sensitivity of the FBAR device. Thus the results in Table 1 clearly show a lower sensitivity for the FBAR than expected. It is hence concluded that the mass transport of the FBAR fluidic system must be the limiting factor.

In Fig. 6 FEM simulations is seen, performed using the COMSOL Multiphysics software to illustrate the flow characteristics over the sensor surface. The flow in the channels was assumed to be of laminar nature and follow the Navier–Stoke set of equations for incompressible fluids. The simulations were made in 2D and for simplicity two orthogonal cross-sections were made in the three-dimensional fluidic system and the corresponding simulations were then performed independently. The pressure difference over the system was set to 0.2 mPa causing the flow rate to be in the range of the one used experimentally.

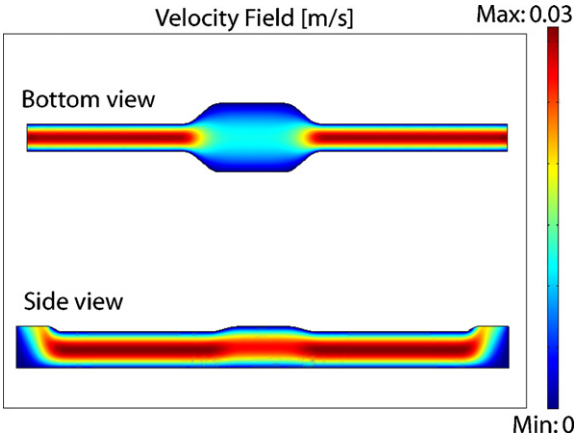

Fig. 6. FEM simulation of the velocity field (m/s) distribution in the micro-fluidic channel.

Under the assumption that the flow is laminar the particle velocity at the walls is zero and within a small distance from the walls the liquid can be considered to be more or less stagnant causing the transport of species to the surface to be manly diffusion driven. It is clearly seen that the majority of the analyte solution passes by the sensor with high speed far from the sensor surface compared to the extension of the active area. The lateral dimension L of the FBAR sensor surface is 300 μm, which is exactly equal to the height H of the cavity underneath. This results in an L/H ratio of 1. This ratio is extremely important for sensor operation in the transport limited regime as it together with the velocity and the diffusion constant of the target analyte determine the efficiency of the transport system. This aspect ratio is highly optimized in commercial QCM systems. Thus, the QCM resonator used in the above experiments had an area of 28 mm^2 and a cell volume of 3 μL resulting in an L/H aspect ratio of 50. This indicates that the design of the micro-fluidic system of the FBAR sensor is highly inefficient and most of the analyte is wasted, that is, a major fraction of the analyte is exhausted without contributing to the detection process. Hence, the measured mass sensitivity is much lower than the theoretical one.

In conclusion it can be said that for realizing the full potential of FBAR biosensors, that is, for achieving their much higher sensitivity special care in designing the micro-fluidic system has to be taken. The results above indicate that in a non-optimized FBAR design the loss of sensitivity can be substantial.

Acknowledgements

This work is supported by the Swedish Agency for Innovation Systems foundation (Vinnova), Sweden as well as by the EU Biognosis project. The technical support of Biosensor Applications AB is kindly acknowledged.

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Biographies

Gunilla Wingqvist is a PhD student at the Solid State Electronics Department, Uppsala University, Sweden, where she has been studying thin film piezoelectric resonator sensors since 2004. In 2003, she received her MSc in applied physics and electrical engineering at Linköping University, Sweden. Her master thesis project was concerning sputter deposition and characterisation of perovskite ferroelectric thin films and was performed at the Thin Film Division at the Department of Physics and Measurement Technology at Linköping University. Her current research involves development, fabrication and characterisation of the AlN thin film piezoelectric resonator for various sensor applications, mainly biosensors.

Johan Bjurström was born 1974 in Timrå, Sweden. He is currently a senior researcher at the Solid State Electronics Department, Uppsala University, Sweden. His research interests include thin film growth of piezoelectric and ferroelectric materials, design and modelling of electroacoustic devices. He received his MSc in material engineering at Uppsala University, in 2002 and earned his PhD degree in engineering science with specialization in electronics and electroacoustics in 2007. Dr. Bjurström's current research focuses on thin film growth and development of thin film acoustic wave devices for sensor applications and frequency control.

Ann-Charlotte Hellgren is PhD in organic chemistry at Uppsala University in 1985. Over 15 years of experience as research and project leader in the field of applied surface chemistry at the Institute for Surface Chemistry (YKI) in Stockholm. Ann-Charlotte Hellgren is manager of the Chemistry Department at Biosensor Applications since August 2000.

Dr. Ilia Katardjiev is an associate professor at the Angstrom Laboratory, Uppsala University, Sweden. His research interests are in the area of ion-solid interactions, thin film physics, microelectronics as well as electroacoustics. Among his greatest achievements is the development of the theory of surface evolution as well as the identification of the sputter-yield amplification effect. He is the director of a number of Swedish and European research programs and leads a group with broad R&D activities in thin film electroacoustic applications, most notably passive and active microelectronic components, chemical and biochemical sensors, etc. Dr. Katardjiev earned his PhD in electrical engineering from Salford University, U.K. in 1989 and since then has been working as a research scientist and a lecturer at Uppsala University. He has published over 100 refereed papers and presented over 20 invited and plenary lectures.