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Short communication

On the applicability of high frequency acoustic shear mode biosensing in view of thickness limitations set by the film resonance

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ABSTRACT

The IC-compatible thin film bulk acoustic resonator (FBAR) technology has made it possible to move the thickness excited shear mode sensing of biological layers into a new sensing regime using substantially higher operation frequencies than the conventionally used quartz crystal microbalance (QCM). The limitations of the linear range set by the film resonance using viscoelastic protein films are here for the first time addressed specifically for FBARs operating at 700 MHz up to 1.5 GHz. Two types of protein multilayer sensing were employed; one utilizing alternating layers of streptavidin and biotinated BSA and the other using stepwise cross-linking of fibrinogen with EDC/NHS activation of its carboxyl groups. In both cases the number of protein layers within the linear regime is well above the number of protein layers usually used in biosensor applications, further verifying the applicability of the FBAR as a biosensor. Theoretical calculations are also presented using well established physical models to illustrate the expected behavior of the FBAR sensor, in view of both the frequency and the dissipation shifts.

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1. Introduction

The use of bulk shear mode electroacoustic resonators for sensing in viscoelastic media, employing polymers or biomolecular species as specific recognition layers for various target molecules, has generated substantial interest in recent years. A typical device is the so-called quartz crystal microbalance (QCM) operating with a resonance frequency of about 5–20 MHz. Recently, the thin film electroacoustic (TEA) technology has made it possible to fabricate quasi-shear mode thin film bulk acoustic resonators (FBAR), operating with a sufficient electromechanical coupling for use in liquid media at 1–2 GHz, made of polycrystalline AlN (Bjurstrom et al., 2006) or ZnO (Gabl et al., 2004) films. The main advantage of the TEA technology is its IC-compatibility, which is a prerequisite for fabrication of sensors and sensor arrays integrated with the electronics, and hence low cost mass fabrication of miniature sensor systems. FBARs often have at least a 1000 times smaller volume and operate at a 100 times higher frequency than QCM. The higher frequency and smaller size results in higher sensitivity. Further, the higher frequency and the smaller size cause all boundary conditions to affect the FBAR operation more strongly than QCM. Therefore, the noise level increases as well, moderating somewhat the huge gain in sensitivity. So far, only FBAR measurements with a network analyzer have been reported in the literature, demonstrating a mass resolution of the same order if not better than that of oscillator based QCM sensors(Weber et al., 2006; Wingqvist et al., 2007, 2008), suggesting that FBARs may be a competitive and low cost alternative to QCM.

The subject of this study is to explore the operation of FBARs in combination with viscoelastic protein films in viscous media, with emphasis on the *detection distance* in relation to the *decay length* and the *film resonance* behavior, which can result in a notable reversed frequency shift. Further, certain limitations stemming from the thickness of the biochemical layers are also studied.

2. Materials and methods

It is common to model the biochemical layers in the same way as polymers, i.e. as homogenous layers obeying the Kelvin–Voigt description of viscoelastic material, in which the complex dynamic modulus (G) can be described as

$$G = G' + iG'' = \mu + i\omega\eta, \quad (1)$$

where G' and G'' are the storage and the loss moduli, respectively. μ is the modulus of elasticity, η is the viscosity and ω is the angular frequency. Further, μ and η are assumed to be real and constant, causing the storage modulus (G') to be constant and the loss modulus (G'') to be frequency dependent.

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In a FBAR, where the frequency of operations is about 100 times higher than for a QCM, the viscous properties of the various layers and media are expected to have a much higher influence on performance, even if the modulus of elasticity and viscosity are assumed to be frequency independent. This is verified by calculations using an analytical model (Voinova et al., 1999). In the parameter space defined by viscosity and elasticity values derived by QCM-D for streptavidin layer (Larsson et al., 2003), it is seen that the frequency shift for QCM depends almost equally on both elasticity and viscosity, while in the FBAR case the frequency shift depends almost exclusively on viscosity. This would already at this point indicate that by combining FBAR and QCM measurements, new information about the acoustic properties of the biochemical layers involved could be obtained.

In the FBAR case one needs to consider also the limitations stemming from the higher frequency and shorter decay length, which are illustrated below. Fig. 1a shows theoretical calculations of the frequency shift of a 1.5 GHz AlN FBAR containing an added viscous layer as a function of the viscous layer thickness for two different such layers. Specifically, calculations according to the analytical model of Voinova et al. are presented along with one-dimensional simulation with the Nowotny–Benes model (Nowotny and Benes, 1987) as well as employing the concept of effective thickness calculated from the amplitude distribution in the system calculated with the Mason model (Rosenbaum, 1988). Fig. 1b illustrates calculations using the analytical formula by Voinova et al. only.

Fig. 1 illustrates with three different theoretical models the expected behavior of sensing of acoustically thick viscous layers.

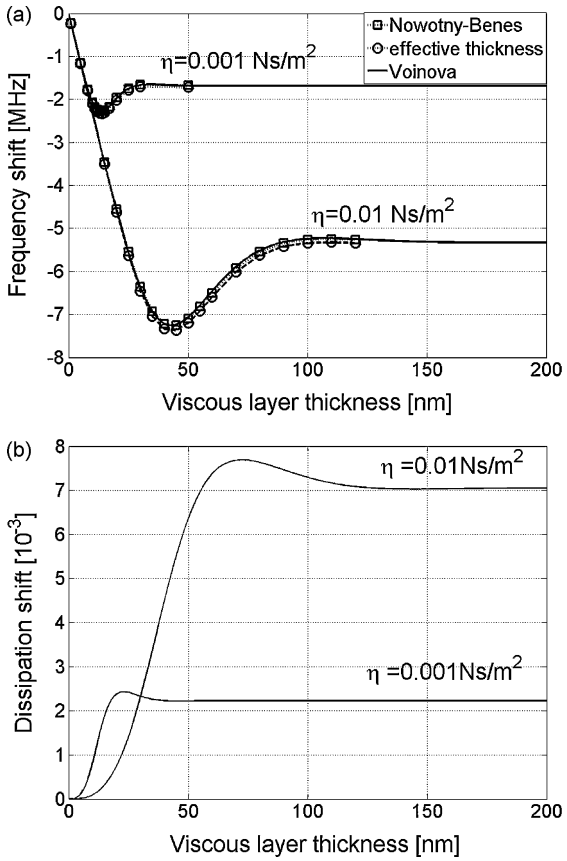

Fig. 1. Calculated frequency shifts (a) and dissipation shift (b), for two 2 μm composite AlN resonators operated in air having imaginary thin electrodes and each having an additional liquid layer of varying thicknesses on one side. The two liquid layers have the same density of $\rho = 997\ kg/m^3$, but different viscosities, $\eta = 0.001\ N\,s/m^2$ and $0.01\ N\,s/m^2$, respectively.

Fig. 1a clearly shows the occurrence of a reversed (positive) frequency shift after which its absolute value decreases steadily to finally saturate as a function of layer thickness. Adding the effect of the surrounding bulk liquid often present in bio-sensing application will affect somewhat the frequency shift behavior in Fig. 1. In addition, the inclusion of a limited elasticity will further affect the level of the saturated frequency shift for thick layers, although not so much the position of the maximum absolute frequency shift. This reversed frequency shift Martin et al. earlier introduced to be caused by the *film resonance* for thick viscoelastic polymer films on QCM, which occurs in the vicinity of a phase shift of $\pi/2$ (that is, layer thicknesses of quarter wavelength) (Martin and Frye, 1991). Around this thickness the constructive interference of the waves inside a low acoustic impedance layer is the strongest, causing the effective layer thickness to be the largest. Note that in the region of positive frequency shift the sensor might also respond with a positive frequency shift to a small increase in layer density, therefore, unaware operation within this region could result in serious misinterpretation of the data.

Further it is seen in Fig. 1b that the behavior of the dissipation versus viscosity differs dramatically between thin and thick layers.

As seen the constructive reflection within the sensed layer will strongly limit the linear range of the sensor. The true detection distance of a real biochemical sensor will be dependent on the actual acoustic properties of the viscoelastic layers involved as well as on the surrounding liquid media. Taking parameters presented by Larsson et al. (2003), the layer thicknesses at which the reversed frequency shift theoretically occurs for a 1.5 GHz FBAR operated in water is calculated to be 120 nm for streptavidin and 40 nm for DNA, respectively. The influence of the electrodes here is considered to be negligible.

In order to study the FBAR effective detection length, multilayer films was deposited in a layer-by-layer fashion onto the sensor surfaces. Specifically, multilayer protein sensing utilizing alternating layers of streptavidin (SA) and biotinated BSA (bt-BSA) was performed. A second multilayer system using the concept of cross-linking fibrinogen with EDC/NHS activation of its carboxyl groups was also studied. The multilayer systems were grown continually while monitoring at the same time the resonance frequency and *Q*-value without flow interruption. A commercial QCM setup (Attana 100 biosensor) has also been used to perform reference measurements and to verify the protocols.

2.1. Device fabrication

Shear mode FBAR sensors were fabricated by sputter deposition of an AlN polycrystalline film with a tilted *c*-axis sandwitched between bottom and top Al electrodes, directly onto a silicon wafer. Silicon micromachining was used to create a micro-fluidic channel system underneath the active area for transporting the liquid to the sensing bottom electrode. Finally, Ti and Au layers were evaporated onto the bottom sensor surface providing identical functionalization conditions as those at the QCM sensor surface. For further details about the fabrication see Bjurstrom et al. (2006).

2.2. bt-BSA—streptavidin multilayer

To achieve a stable multilayer the highly specific interaction between SA and biotin was used by alternating injections of SA and bt-BSA. SA was prepared at 100 μg/mL in PBS and bt-BSA was prepared at 10 μg/mL in phosphate buffered solution (PBS). The assumed growth behavior of the multilayer is illustrated in Fig. 2a.

First, the bt-BSA was injected and adsorbed onto the sensor surface by halting the flow of 10 μg/mL of bt-BSA for half an

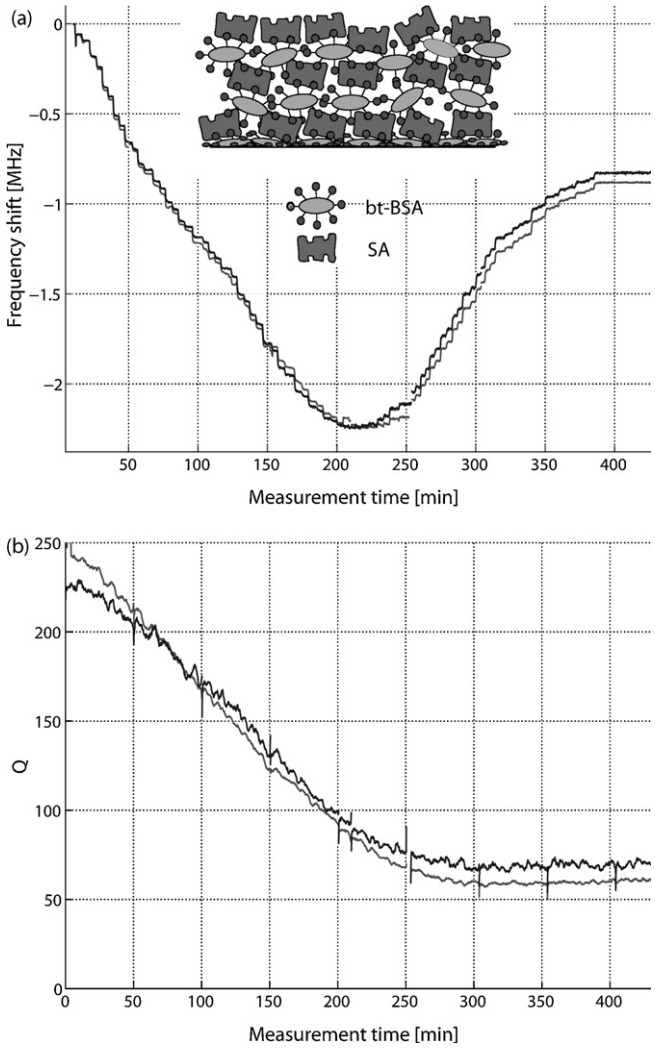

Fig. 2. Measured frequency shift (a) and Q values (b), during stepwise injection of alternating strepavidin and biotinated BSA to a 800 MHz FBAR. The two curves in each figure correspond to the series resonance (grey) and the parallel resonance (dark grey).

hour to prepare the surface. Subsequent injections of SA and bt-BSA were then performed in an alternating manner at constant flow.

2.3. Fibrinogen with EDC/NHS activation multilayer

A two-step cycling protocol involving activation with EDC/NHS and coupling chemistry with fibrinogen was optimized utilizing null ellipsometry and QCM to ensure accurate thickness and uniform distribution of each layer over the surface. Details can be found in Lennartsson (2007).

3. Results and discussion

3.1. bt-BSA—streptavidin multilayer

Initial QCM measurements confirmed that the chemical reactions are very stable for at least 100 SA/bt-BSA layers (that is 200 injections), where the frequency shift standard deviation was 22 and 12 Hz for the SA and bt-BSA, respectively, ensuring no collapsing of the biochemical multilayer within the FBAR experiment. A well controlled reference measurement was then performed with the QCM. In the QCM reference measurements each bt-BSA injection resulted with good repetitivity in a 60 Hz frequency shift, while the SA injection resulted in a frequency shift between 100 and 115 Hz. The measured SA shift agrees well with the calculated frequency shift, using the Voinova model for the following parameter values $\rho = 1060$, $\mu = 0.28$ MPa and $\eta = 82$ mPa s according to Larsson et al. (2003).

Subsequently, up to 90 injection steps were employed in the FBAR experiments. Fig. 2 shows the results from the FBAR measurements with SA/bt-BSA multilayers. The long duration of the experiment required several re-probing to ensure good contact, causing some irregularities as seen in the curves. The experiment illustrates the reverse frequency shift, which in this case occurs at around 20 SA/bt-BSA layers. The resonance frequency becomes insensitive to film thickness at around 40 SA/bt-BSA layers. The frequency response is weaker than expected from the calculations above while the Q value is higher than expected. The FBAR sensor experienced some electrode degradation during the measurement due to non-optimized electrode configuration, therefore, there might be some uncertainties in the absolute values presented but the trend is still clearly illustrated. The series resistance did only vary between 1 and 4 ohms and could mainly be correlated to the probing. The experiment was also repeated with FBAR sensors with thinner AlN, at operating frequencies of 1.0 and 1.2 GHz, which showed the reverse frequency shift to occur at less number of layers with increasing frequency, as expected from the theory. From the size of the molecules, each SA/bt-BSA pair of layers was roughly estimated to be about 10 nm thick (Cassier et al., 1998; Hook et al., 2002), causing the thickness of the protein layer, at which reversed frequency shift occurs, to be between 100 and 200 nm for the tested FBARs.

3.2. Fibrinogen with EDC/NHS activation

The thickness increase of the fibrinogen layer for each cycle as measured with null ellipsometry was found to be about 3 nm. The point of reversed frequency shift occurred at a number of protein layers of about 10–20 corresponding to 30–60 nm for FBAR sensors operating from 1.2 GHz down to 700 MHz. Certain differences in the QCM and FBAR response were observed, especially during the EDC/NHS activation step, which could be due to the different sensing behaviors set by the very different frequencies (e.g. see specifically the loss modules in Eq. (1)). Further measurements are, however, necessary to provide a full explanation of these differences.

4. Conclusion

Even though the effect of reversed frequency shift around the film resonance using viscous or viscoelastic films is known, it is seldom addressed in the case of QCM since very few applications operate at this limit. With respect to FBARs which operate at much higher frequencies this effect needs to be studied specifically in order to verify the range of applicability of FBARs and other high frequency acoustic shear mode devices as biosensors.

The linear range has been studied experimentally for FBAR sensors operating from 1.2 GHz down to 700 MHz with both alternating injections of SA and bt-BSA and by stepwise cross-linking fibrinogen with EDC/NHS activation of its carboxyl groups. The point of reversed frequency shift occurred at a number of protein layers of about 10–20, for the fibrinogen layer, which was defined by ellipsometry to correspond to 30–60 nm. The point of reversed frequency shift was at about 10–20 for the double protein SA/bt-BSA layers, which from the size of the protein molecules was indicatively estimated to 100–200 nm. The results indicate a safe underestima-

tion of the linear range to 20 nm, but could very well be much larger for some protein systems.

In both cases the number of layers required to reach the regime of reversed frequency shift caused by the film resonance is well above the number of 1–4 protein layers usually used in biosensor applications. The limit imposed by the film resonance will be of much greater significance in the case of gas sensing employing thick polymers, even though such polymers usually exhibit higher acoustic impedance than the protein layers described here. With respect to FBAR biosensor applications this phenomenon is expected to play a role in some applications where long DNA strains are used, which are proven to have a rather low viscosity (Larsson et al., 2003) and are, therefore, expected to be acoustically long molecules.

It is further noted that additional FBAR measurements of the biochemical systems studied above could be used to extract acoustic properties of the protein layers. Such measurements compared to QCM measurements could provide new information about frequency dependence of properties, such as viscosity and elasticity.

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