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Monolithic Fabrication of Wireless Miniaturized Quartz Crystal Microbalance (QCM-R) Arrays and their Application for Biochemical Sensors

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We report on a novel wireless quartz crystal microbalance sensor array with integrated microcoils for remote readout (QCM-R). It consists of miniaturized quartz crystal microbalances solely fabricated by microfabrication batch processes including wet chemical thinning of quartz membranes, electroplating of microcoils through lithographic masks and via interconnections through the quartz substrate. Measurements with varying distance between the QCM-R and readout coil showed the resonant frequency independence of the coupling. Experiments with immobilized protein A and the adsorption of its antibody proved the excellent performance of the system.

1. Introduction

Mass sensitive transducers utilizing thickness shear modes (TSM) are increasingly studied for immunosensor application.[1–3] These devices typically consist of a thin AT-cut quartz wafer with electrodes patterned on opposite sides, which correspond to the mass sensitive area. The immunological receptors (antibodies or antigens) are immobilized on these electrodes. The resonant frequency f_0 of the free resonator is established when a standing transverse acoustic wave condition is fulfilled. It is related to the crystal thickness t_s following eq. (1)

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$$f_0 = \frac{\sqrt{\mu_q / \rho_q}}{2t_s} \tag{1}$$

Here, μ_q and ρ_q are the shear modulus and the density of quartz, respectively. The dependence of the resonant frequency on the thickness enables the determination of mass changes in the thin immunological films at the resonator surface. For the application as an immunosensor, the quartz resonator is used as the frequency determining component in an oscillator circuit. The measuring signal is the resulting frequency shift Δf due to the mass deposition Δm of an analyte on the surface. Sauerbrey[4] first derived eq. (2) for Δf where A_{piezo} is the piezoelectrically active area.

$$\Delta f = -\frac{2 f_0^2 \Delta m}{A_{piezo}\left(\mu_q \rho_q\right)^{1/2}} \tag{2}$$

For operations in liquids, the resonant frequency also depends on the viscosity η_L and density ρ_L of the liquid the crystal is immersed in. The theoretical frequency shift for the viscosity-density effects without additional mass loading is given in eq.(3).[5]

$$\Delta f = -f_0^{2/3} \sqrt{\frac{\eta_L \rho_L}{\pi \mu_q \rho_q}} \tag{3}$$

For operating a coated resonator in a liquid, eqs. (2) and (3) have to be superimposed. Figure 1 shows the equivalent circuit of a coated resonator in liquids.[6] The inductance L corresponds to the energy storage in the vibrating quartz mass, the capacitance C is related to the mechanical elasticity and the resistance R contains the energy losses due to the various processes. The dielectric quartz substrate between the electrodes forms a static capacity C_p.

The liquid loading affects an additional inductance and resistance, whereby L_1 relates to the vibrating liquid mass and R_1 to the viscous losses. This model is only valid for one side contact of the quartz with the liquid. Otherwise another parallel conductance and capacitance have to be added. For biochemical applications in liquids a sensitive coating is added, resulting in another complex impedance Z_m. For thin rigid layers this complex value is reduced to an inductance, here named L_m.

Apart from a wireless SAW-sensor system[7] and a macroscopic discrete wireless QCM-setup,[8] there is no integrated approach to the presented device, to the authors' knowledge. Our realized new miniaturized wireless sensor system offers the advantages of reduced fabrication and packaging cost independent of the readout circuitry. This enables the use of sensitively coated QCM-R in dangerous environments where the sensor is exposed to toxic or contagious media in hermetically sealed measurement setups. Addi-

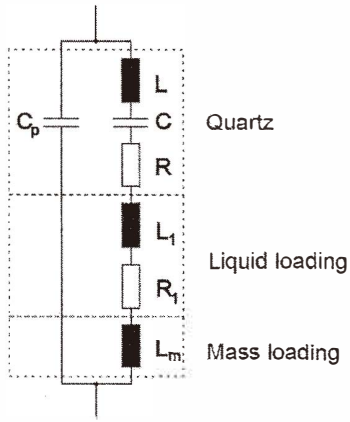


Fig. 1. Equivalent circuit of a coated QCM in liquid.

tionally, disposable or exchangeable sensors and sensor arrays can be used with reusable readout circuitry which is of major interest for biotechnological or pharmacological screening in micro titer plates.

2. Device Fabrication

2.1 *Integrated array*

We have previously reported on the miniaturization of quartz resonators and arrays.[9,10] The most important process steps for the fabrication of QCM-R are shown in Fig. 2. AT-cut quartz blanks were obtained from Industria Elettronica Varese, Italy. The lateral dimensions are 38.1×38.1 mm^2, the thickness is 128 μm, and both surfaces are polished. Prior to processing, the quartz blanks are cleaned in a mixture of sulphuric acid and hydrogen peroxide and rinsed in deionized water. The quartz etching solution consists of a mixture of water, hydrofluoric acid, and ammonium fluoride and is heated to 60°C under constant stirring.

After sputter deposition of Cr/Au mask layers, holes for the via interconnections are opened by photolithography. The quartz material is then etched to the desired thickness. In the next step, the membrane areas are etched and at the same time the via interconnections are etched through the blank. By controlling the etch time, we reach resonant frequencies of the QCM between 13 and 50 MHz. The resist and metal layers are then stripped and new Cr/Au films are deposited. These are patterned to form the QCM electrodes.

We first used a standard positive photoresist. The problems occurring with this technology can be seen in Fig. 3(a). The membranes are almost completely filled with resist while the unetched areas are covered with a thin resist film only. These differences make the development step very difficult, since there either remain resist residues in the edges or the resist is completely overdeveloped and removed on the unetched areas.

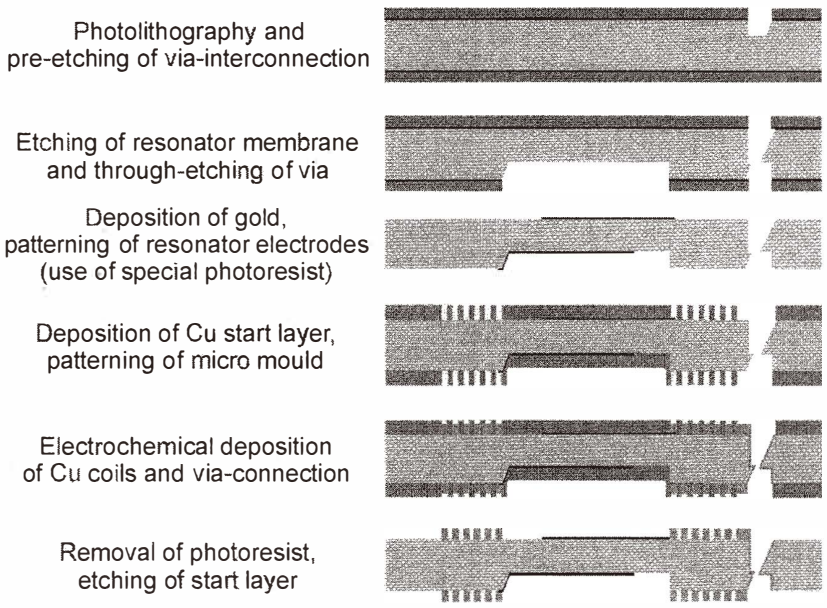
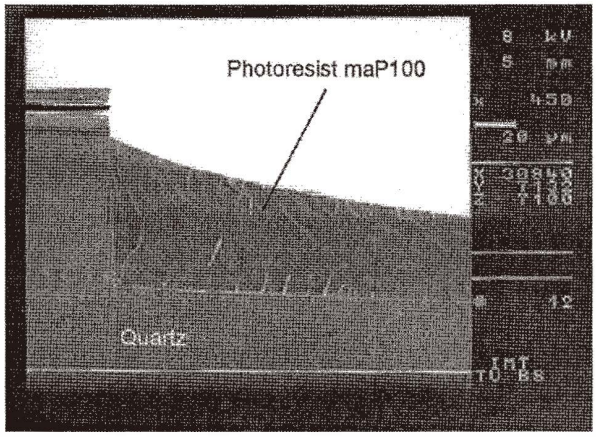


Fig. 2. Process flow for the fabrication of QCM-R.

To overcome these problems, we use an electrophoretically disposable photoresist (PEPR2400; Shipley), as described *e.g.* by Linder *et al.*[11] Applying a voltage of about 120 V between the gold layers on the quartz (anode) and two parallel stainless plates (cathode), a resist film of about 5 μm thickness is deposited in 3 s. The positive tone resist can be developed in a standard alkaline developer. The results of this improved process step can be seen in Fig. 3(b), where very uniform resist layers were deposited onto the unetched part as well as in the membrane area.

Subsequently we pursue the additive fabrication of copper microcoils through positive photoresist microforms on both sides of the quartz chip.[12,13] After completion of the quartz material structuring and patterning of Au electrodes, a Cr/Cu seed layer is sputter deposited on both sides of the wafer. UV-depth lithography is carried out on both sides using AZ4562 or AZ9260 from Clariant for the formation of the coil microform. For spin coating, a closed lid spinning tool is used guaranteeing a highly even and smooth topography with little edge beading even above the etched cavity and via holes. The resist thickness was adjusted to 23 μm which is sufficient for electroplating structure heights of approx. 15 μm with homogenous thickness distribution. After spin coating, the resist is softbaked in an enclosed oven or a hotplate that slowly ramps the temperature to 90°C. This measure reduces skin effects and provides an even solvent concentration throughout the resist. Before exposure, it is essential to let the DNQ-Novolak resist take up water for at least three hours in ambient air which increases sensitivity since the photoreaction of DNQ consumes water. The micro molds for via interconnects and the coil layers are

(a)



(b)

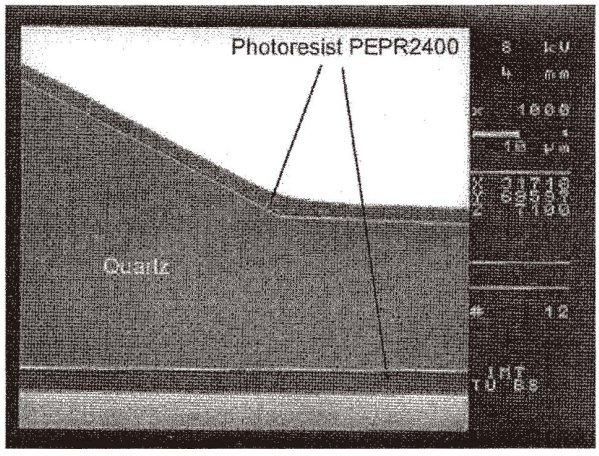


Fig. 3. Improvement of coating uniformity by employment of electrophoretically deposited photoresist.

lithographically patterned separately, to compensate for the much higher resist thickness above the vias. This way a large exposure dose can be used for the vias by maintaining a high-precision pattern quality for the coil microform.

After lithography on both sides, the chip is immersed in a sulfate copper electroplating bath (CuBath SC, Enthone OMI) resulting in simultaneous growth of via interconnects and coil structures on both sides. Subsequently, the resist mold is stripped and the seed layer is wet chemically etched. Figure 4 displays SEM details of the fabricated QCM-R arrays. In the middle, a whole device can be seen from the side of the thinned membrane. Each coil layer is wound around the rim of the membrane on the bulk material and makes contact with the thin film Au electrode on the inner side and the via interconnection on the outer

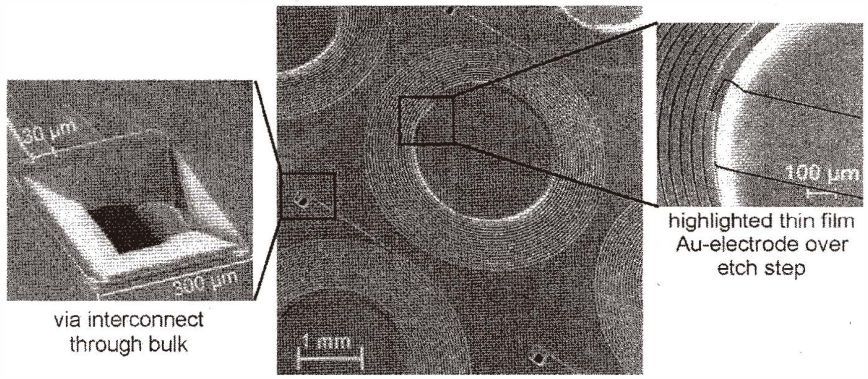


Fig. 4. SEM of a part of the sensor array with enlargements of via interconnection through quartz wafer and connection of resonator electrode with the micro coil. Shown here are three of up to 25 realized via interconnects of the QCM-R arrangements.

side. The magnified details show the via interconnect completely etched through the bulk and filled with electroplated Cu (left) and the thin film electrode over the 100 μm etch step (right) patterned by electrophoretically deposited photoresist PEPR 2400 from Shipley.

2.2 *Readout circuit*

A readout circuit for a passive telemetric QCM-sensor has been developed (see Fig. 5). A voltage controlled oscillator (VCO) is used to excite the sensor via an external (primary) coil over a certain frequency range, while the current in the primary coil is monitored. At the accumulated mass dependent resonant frequency of the QCM / micro (secondary) coil arrangement, the current shows a sudden decrease caused by the extremely low impedance at the series resonance of the quartz resonator. If the transmission line is suitably designed, the overall amplitude and coupling do not influence the frequency behaviour in the range of interest (20–50 MHz). The integration of a frequency counter and a display therefore yields a standalone sensor system, capable of securely measuring the frequency shift caused by a physical or (bio)chemical effect.

3. Experimental

3.1 *Performance of wireless sensor system*

In order to characterize the performance of the QCM-R, we first monitored the impedance of a hand-wound coil, which was positioned close to the QCM-R array. The tests proved reliable resonant frequency detection over ranges up to 12 mm (see Fig. 6). While the signal amplitude decreases with increasing distance due to poorer inductive coupling, the positions, i.e., the frequencies of the maximum and minimum, remain constant. These frequencies correspond to the series and parallel resonant frequency of the

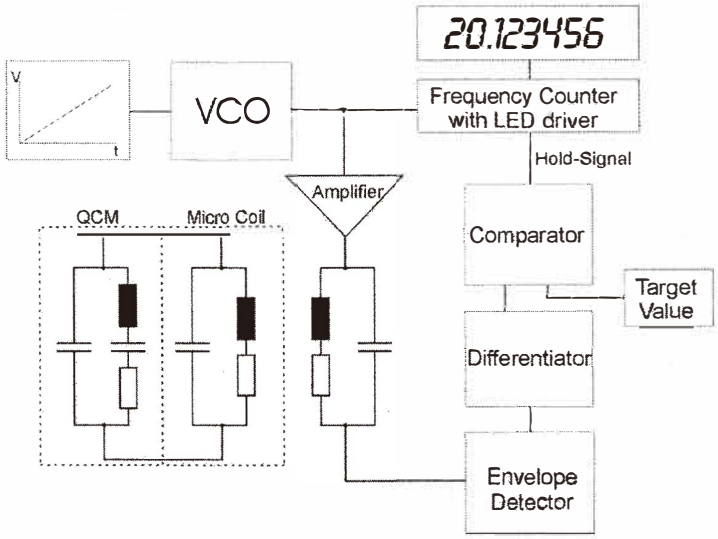


Fig. 5. Block diagram of developed readout circuit.

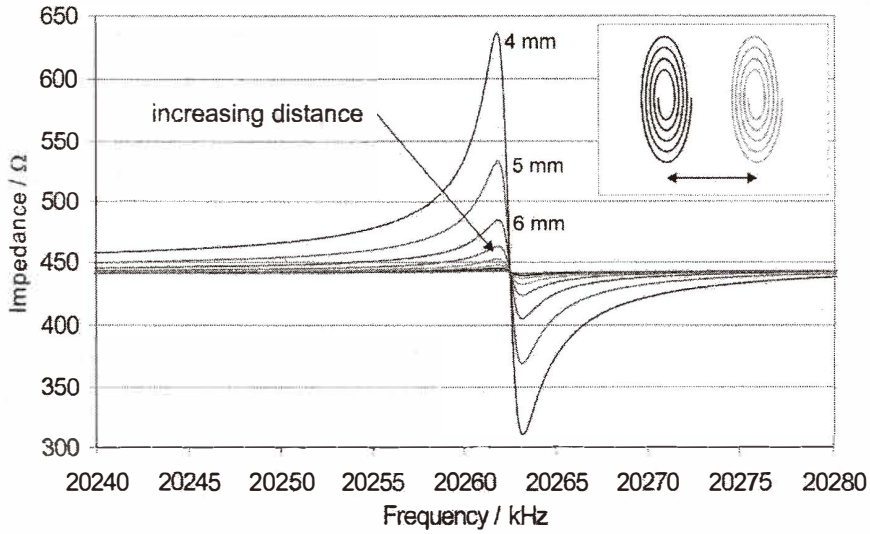


Fig. 6. Network analyzer measurements with varying distance (4...12 mm) between QCM-R and readout coil prove resonant frequency independence of coupling.

quartz resonator, respectively.

Another measurement series analyzed the influence of deviations from the coaxial alignment. For small displacements, the amplitude decrease is very small. However, if the

distance between the coil axes is larger than the coil diameter, the signal is heavily damped (see Fig. 7).

Further experiments were carried out to determine the influence of angle variations of the coils. Up to a misalignment of about 30°, there is almost no influence on the inductive coupling, as can be seen in Fig. 8. If the coils axes are positioned perpendicular to each other, the amplitudes disappear completely since there is no magnetic field permeating the QCM-R coil.

Moreover, the coupling through plastics and glass is possible which allows for the proposed use in dangerous media in hermetically sealed environments. The measurement results are in agreement with theoretical considerations, since the coupling between two coils decreases nonlinearly with increasing distance and misalignment.

3.2 *Immunosensor*

The applicability of the developed system as a biochemical sensor array was investigated using an immunoassay, which takes advantage of the affinity binding between antibodies and the corresponding antigens to detect their presence in a mixture to be analyzed. Other biochemical analysis systems need special labels, *e.g.,* fluorescent, radioactive or electrochemical matters, for the indirect detection of immune reactions. These labels induce a measurement signal which is dependent on the analyte concentration. A quartz crystal microbalance array has label free operation since the resonant frequency shift caused by mass accumulation and/or changes in the surface conditions can directly be detected. For the detection of a certain antigen, a matching antibody has to be immobilised on the sensor surface.

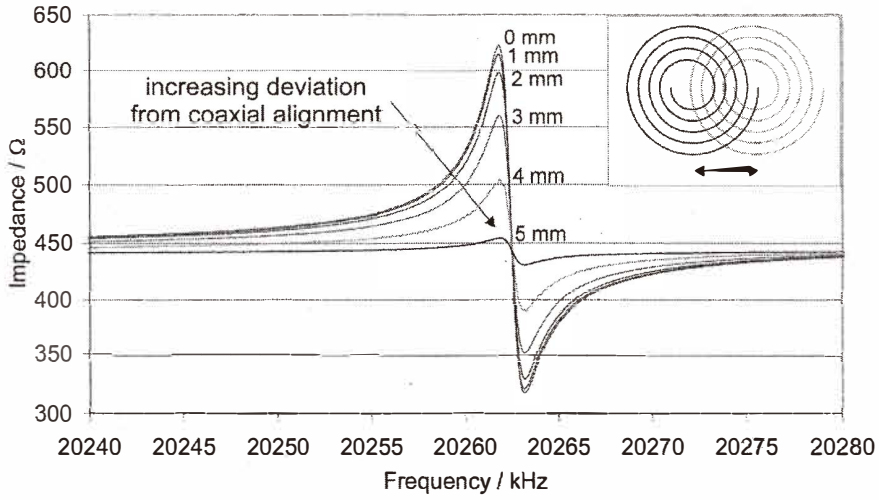


Fig. 7. Network Analyzer measurements with deviations from coaxial alignment. Distance between coils in 5 mm, displacement is varied between 0 and 5 mm for a middle coil diameter 5 mm.

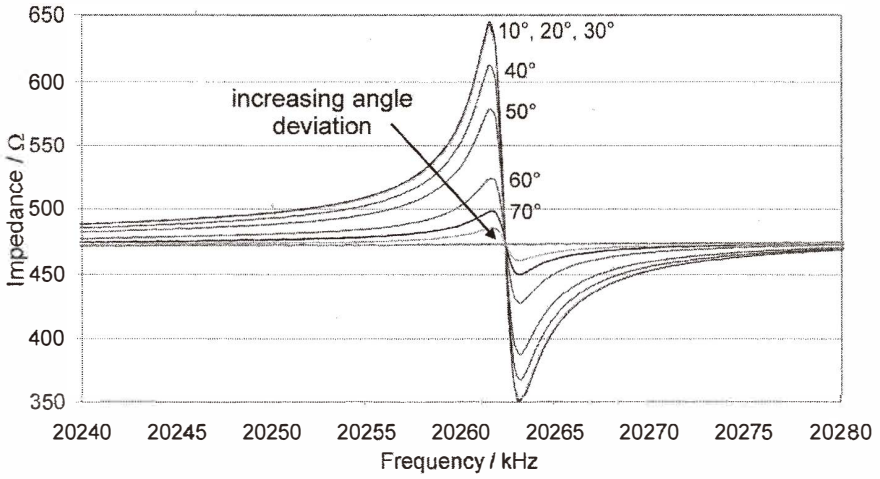


Fig. 8. Network analyzer measurements with deviations from parallel alignment. Distance between coils is 5 mm, and the angle is varied between 10°C and 90°C for a middle coil diameter of 5 mm.

For application in liquids, PVC-reservoirs with a diameter of 3 mm and a volume of approximately 100 μL are fixed on top of each single QCM, in which the different analyte solutions (volume 70 μL) can be pipetted. Thus only the etched membrane areas and not the unprotected copper coils come into contact with the liquid. Up to 25 sensors on one quartz substrate with overall dimensions of 28 mm by 28 mm were fabricated.

We used protein A for the immobilisation and tested the functionality of this undercoating by accumulation of anti-protein A. An important advantage of the QCM technique is the potentiality of online measurements. We monitored the adsorption processes for protein A and its antibody in this way as depicted in Fig. 9. After recording reference values in buffer solution (phosphate buffered saline - PBS) protein A was incubated (concentration 1 mg/ml PBS). The resonant frequency decreases until there is a balance between adsorption and detachment. In the next step, possibly unoccupied sites on the surface, which could influence the following adsorption of anti-protein A, are blocked with bovine serum albumine (BSA - concentration 10 mg/ml PBS). Since there is almost no observable frequency decrease, the protein A layer seems to be very dense.

The incubation of anti-protein A (concentration 1.58 mg/ml PBS) leads to a further strong frequency decrease due to adsorption at protein A. The frequency shift is much larger than that for the adsorption of protein A, which is caused by the higher mass of the anti-protein A molecule. The affinity of anti-protein A was analyzed using a variety of dilutions in a series. Figure 10 displays the results of the frequency shift measurements for a 20MHz resonator array. The mass increase at the QCM is not proportional to the concentration of anti-protein A, which agrees with the theoretical sigmoidal correlation. The sensor arrays are reusable since the bond between protein A and anti-protein A can be opened with diluted sodium hydroxide as was proven by recording of the QCM resonant frequencies during the complete course of the experiment.

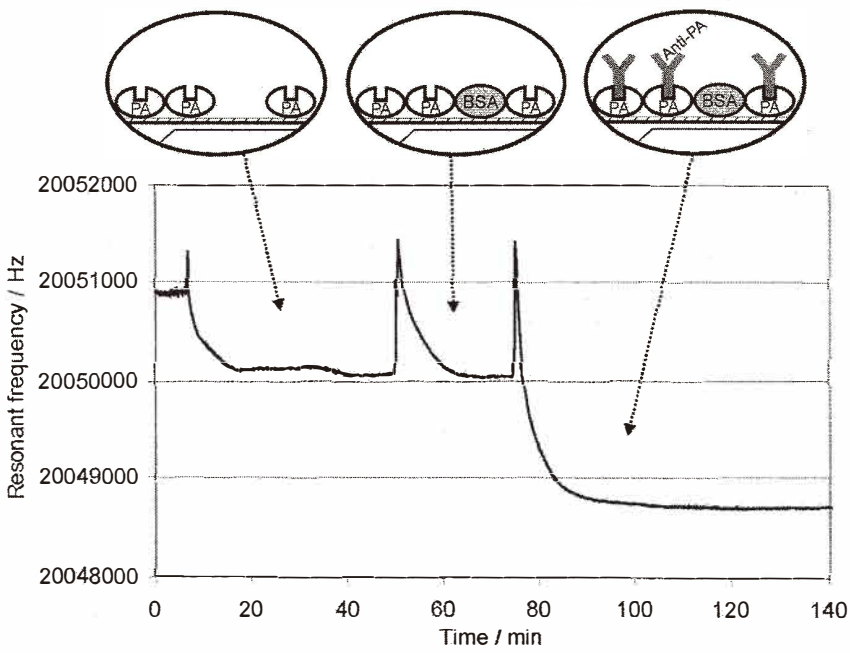


Fig. 9. Frequency decrease due to adsorption of protein A, bovine serum albumine and anti-protein A.

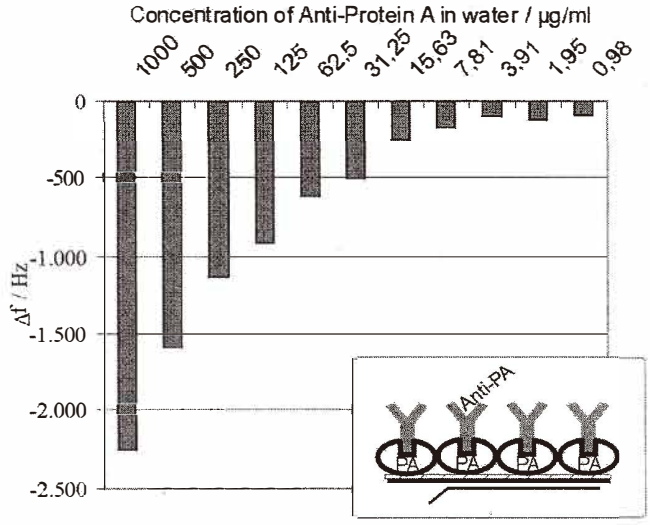


Fig. 10. Dilution series with anti-protein A in water for investigating the affinity to immobilized protein A. The schematic shows the principle of the mass increase.

4. Conclusions

We have demonstrated a new monolithically fabricated miniaturized QCM array for biochemical sensing with the additional capability of wireless resonant frequency readout. The latter is useful for measurement in enclosed environments and is cost effective for packaging and the reuse of electronic readout circuitry after discarding of the sensor array. The miniaturization increases measurement frequency as well as sensitivity and allows for the minimal necessary amount of analyte, which can be essential *e.g.* for the monitoring of expensive substances. The integration on a single chip simplifies the temperature control during measurements. Since immune reactions for diverse samples can be monitored simultaneously, the technique is time saving. Additionally, reference measurements which are always necessary can be performed using the same setup.

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