Sensors
October 2009, Volume 1-3, pp. 687 - 690
http://dx.doi.org/10.1109/ICSENS.2009.5398346

A biosensor for detection of DNA sequences based on a 50MHz QCM electronic oscillator circuit

Bustabad, E.A[1].; Garcia, G.[1]; Rodriguez-Pardo, L.[1,*]; Faria, J.[1]; Perrot, H.[2,*]; Gabrielli, C.[2]; Bucur, B.[2]; Lazerges, M.[2]; Rose, D.[2]; Compere, C.[3]; Arnau, A.[4]

[1] Dpto. Tecnologia Electronica Universidad de Vigo Vigo, Spain
[2] IFREMER, Service Interfaces et Capteurs Centre de Brest Plouzané, France
[3] CNRS-LISE Université P. et M. Curie Paris, France
[4] ETSI de Telecomunicación Universidad Politécnica de Valencia Valencia, Spain

* Corresponding author : Rodriguez-Pardo, L., email address : lpardo@uvigo.es, Perrot, H., email address : hubert.perrot@upmc.fr

Abstract:

This work deals with the development of a high sensibility DNA biosensor based on a 50MHz Quartz Crystal Microbalance (QCM) Oscillator Electronic Circuit. The designed QCM oscillator sensor is able to detect the presence of complementary DNAs in a solution that match the sequence on a given strand in function of the changes in the output frequency of the oscillator. The design is adapted so that the Barkhausen condition is satisfied even when the quartz is immerged in liquid media. Also a comparative study of the developed 50MHz biosensor in front of a QCM oscillator with smaller frequency is carried out, with object of checking if the sensibility of the oscillator increases, allowing to detect smaller concentrations of the complementary DNA.

I. INTRODUCTION

Specific DNA sequence detection is a major issue in life science. An important advance in this field was done during the last two decades with the design of DNA biosensors. They are more efficient by comparison to DNA hybridization tests performed on membranes that are less sensitive, less selective, time consuming and not time resolved. DNA biosensors are now intensely developed for diagnostic applications, environmental monitoring and food controls. DNA detection biosensors are based in the hybridization process of combining complementary, single-stranded DNA into a single molecule.

The quartz crystal microbalance (QCM) oscillator circuits are useful to design DNA-biosensors [1]. A QCM sensor typically consists of an oscillator circuit containing a thin AT-cut quartz disc with circular electrodes on both sides of the quartz. Due to the piezoelectric properties of the quartz material, an alternating voltage between these electrodes leads to a mechanical oscillations of the crystal. These oscillations are generally very stable due to the high quality of the quartz (high Q factor). If a mass is adsorbed or placed onto the quartz crystal surface, the frequency of oscillation changes in proportion to the amount of mass. Therefore, these devices can be used as high sensitivity microbalances intended to measure mass changes in the nanogram range by coating the crystal with a material which is selective towards the species of interest. In fig. 1 the structure of a DNA biosensor based on a QCM oscillator is shown. The quartz crystal acts as a signal transducer, converting mass changes due to the hybridization process into frequency changes. One of the main advantages of this device is the ability to control a QCM's selectivity by applying different coatings, which makes this sensor type extremely versatile.

The design of crystal controlled oscillators used as QCM sensors in fluids is a difficult task due to the wide dynamic values of the resonator resistance that they should support during their operations [2]. The piezoelectric quartz experiences a strong reduction of its quality factor due to the increase of the losses (R_Q) caused by the liquid [3]. Fig. 2 shows the BVD equivalent circuit of a piezoelectric resonator modified by Martin and Granstaff for a quartz crystal loaded by the mass of a material layer and a liquid. The standard design of oscillators, as Pierce or Colpitts, does not work well since, although they provide a great stability in frequency and a low phase noise, their gain and phase are very sensitive to the losses of the resonator [4]. A good design of a sensor oscillator for liquid media will maintain the necessary loop gain and phase for the oscillation in a wide margin of values of the loss resistances of the quartz.

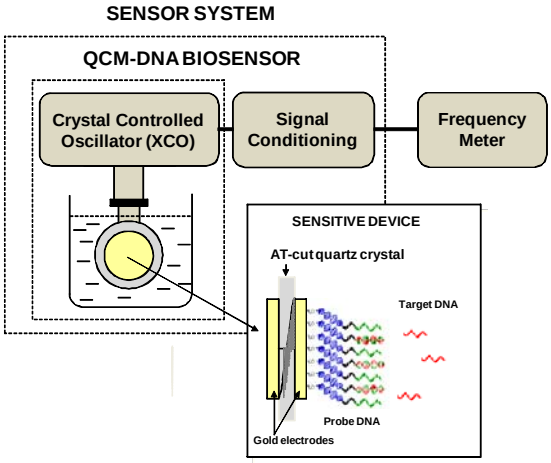

Fig.1 Diagram of a QCM oscillator circuit used as biosensor for DNA detection

ANR PRECODD (HAB SEACHIP) is gratefully acknowledged for financially supporting this research.

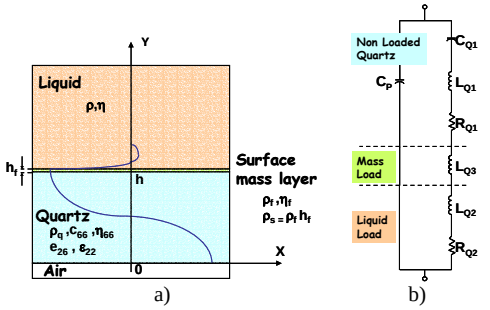

Fig.2. Electromechanical model of a piezoelectric resonator for microgravimetrical applications in liquid; a) Cross-section of a loaded resonator b) BVD equivalent circuit

This work deals with the design and implementation of a 50MHz QCM electronic oscillator circuit for its use as high sensitivity DNA biosensor. The QCM oscillator sensor is able to detect the presence of complementary DNAs in a solution that match the sequence on a given strand in function of the changes in the output frequency of the oscillator. The design is adapted so that the Barkhausen condition is satisfied even when the quartz is immerged in liquid media. An experimental characterization of the frequency stability of the oscillator is carried out, with object of determining the resolution of the sensor. The behavior of the oscillator as DNA biosensor is proven, by monitoring its frequency during the process of immobilization of probe DNA on the gold-covered quartz surface of the 50MHz QCM oscillator and during the hybridization of complementary target DNA present in a solution. Also, a comparative study of the developed 50MHz biosensor in front of a 27MHz QCM oscillator is carried out with object of checking if the sensibility of the oscillator increases, allowing to detect smaller concentrations of the complementary DNA. Finally, a calibration of the DNA biosensor with buffer solutions of different target DNA concentrations is carried out and the minimum concentration of DNA detectable is determined.

II. THE 50MHZ QCM OSCILLATOR SENSOR

To design the quartz crystal oscillator circuit for its use as QCM sensor in liquid media, Miller oscillator topology was chosen. The Miller topology is a high-frequency stable topology that allows designing sensors of high resolution [5-7]. This topology, in spite of not being the most adequate topology for obtaining the best frequency stability [8], experimentally showed a good capacity to work under strong damping [9, 10]. Miller oscillators allow solving the problems that have the standard oscillators to work in liquid, as Colpitts or Clapp, thanks to its ability of supporting a wide range of values of the resonator resistance due to the damping.Once decided the configuration, the design and simulation of the circuit was done with the help of Orcad PSpice. To model the quartz resonator, the experimental values of the parameters of the equivalent electric circuit in distilled water were used. They are summarized in table 1. The Burr–Brown OTA660 transconductance amplifier was chosen as the active device [11]. To

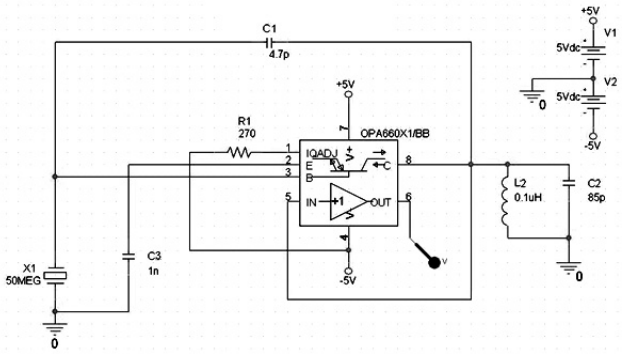

Fig.3. Schematic of the 50MHz Miller oscillator circuit

determine the values of the components, the design considerations for this topology in [6, 7] were realized. The OTA was polarized using a resistance of 220 ohms to have a high gain. In fig. 3 a simplified scheme of the designed oscillator is shown.

TABLE I. EXPERIMENTAL VALUES OF THE BVD EQUIVALENT CIRCUIT FOR THE QUARTZ IN AIR AND IMMERGED IN DISTILLED WATER

	R_q[Ω]	L_q[μH]	C_q[fF]	C_p[pF]	f_s [Hz]	Q
Air	44.8	2330	4.34	10.7	50049219.2	16351
Water	130	2349	3.34	17.9	49841134.0	5660

Once the oscillator circuit was designed and simulated with PSpice, it was implemented on a printed circuit board (PCB). All the elements were included in the PCB with exception of the quartz crystal, which was connected to the circuit using a BNC connector. A temperature sensor was also incorporated to externally control the temperature of the circuit by means of a WATLOW regulator. In fig. 4a a picture of the implemented PCB is included.

In order to be able to immerse the grounded side of the quartz resonator, the quartz was placed on the support pictured in fig. 4b. A conductive silver paste was used to carry out the contact between the quartz electrodes and the cables located in the other side of the support. To only leave uncovered the electrode of the crystal that will be used as DNA sensor and to isolate the contacts and the other face of the crystal, a silicon layer was applied. The group quartz-support is placed inside a plexiglass cell that includes a BNC female connector to which the cables of the support are soldered. This cell is connected to the corresponding BNC male connector incorporated in the metallic box in which the PCB is located with the rest of the oscillator (see fig. 4.c).

Once implemented the electronic oscillator circuit, it was experimentally characterized in the measurement environment. The output frequency of the oscillator was connected to a Fluke PM6685 frequency counter controlled by a software program that allows storing the frequency samples. The temperature of the electronic circuit was controlled by a Watlow regulator. Experiments were made with the plexiglass cell (and therefore the quartz and its environment) included in a BMT Climacell climatic cell which allows maintaining

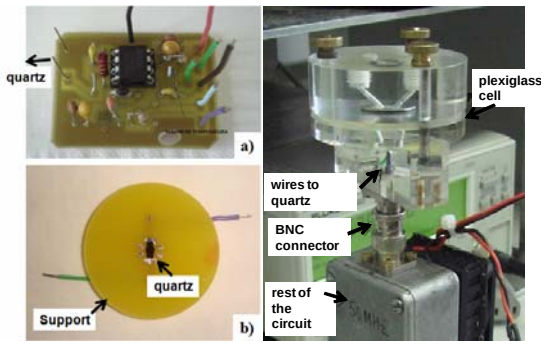

Fig.4. Implementation of the sensor system a) PCB b) Support with quartz. c) Connection of the cell with the quartz to the rest of the oscillator

constant the ambient temperature and humidity. A micropump was used to provide a constant flow of liquid circulating over the surface of the crystal. The flow rate was chosen low (50μL/min) to minimize noise in the quartz. In order to characterize the designed system, a study of the frequency stability of the oscillator was carried out by means of the Allan deviation $\sigma_y(\tau)$[12]. Allan deviation characterization is commonly used because it allows the determination the stability of an oscillator in a time interval,τ, for a certain application. The oscillator detection limit, i.e. the smallest frequency deviation that can be detected in presence of noise is equal to [2] $\Delta f_{noise}(\tau) = \sigma_y(\tau) \cdot f_o$, where f_0 is the nominal frequency. In QCM applications, the mass resolution can be obtained by the relationship between the detection limit and Sauerbrey sensitivity of the sensor by $Resolution = \Delta f_{noise}/k$, where $k = 2.26 \cdot 10^{-6} \cdot f_o^2$ (Hz g^{-1} cm^2) is the mass sensitivity coefficient, known as the Sauerbrey coefficient [13].

A detection limit of 40Hz was found in the system when the oscillator was used as DNA biosensor. Therefore the designed system has a mass resolution of 7.1 ng/cm^2.

III. THE DNA BIOSENSOR

A disulfide-DNA biosensor was designed using the 50MHz QCM oscillator by immobilization of a 20-base DNA-disulfide probe A in NaCl solution on the gold quartz surface. The structure of DNA strands used is presented on fig. 5, and the immobilization process is illustrated in fig. 6 (1). The solvent for DNA immobilization was 0.5 M NaCl referred to as "NaCl". Immobilization of the recognition element on the surface of the transducer, in our case the DNA-disulfide probe A, is a key stage in the construction of a biosensor. The covalent union of the probe DNA with the gold electrode of the quartz takes place thanks to that the used concentration of DNA contains sulfur (S) that will carry out the union between the gold of the electrode and the DNA. To carry out the immobilization the concentration of DNA is added to the NaCl solution and a 50 μL/min constant flow of this solution is maintained in the

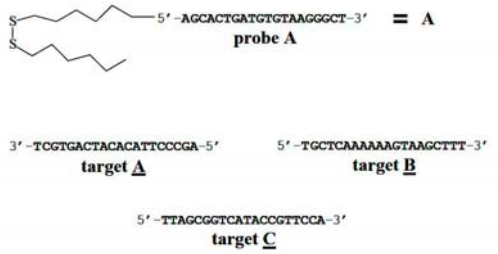

Fig.5. DNA strands structures

plexiglass cell in which is the quartz resonator is included.

After the immobilization, DNA-disulfide probe A was hybridized in HEPES solution with a complementary DNA target A (2). A/A are 20-base complementary sequences (fig. 5). Hybridization experiments were performed in 0.05 M HEPES, with 0.5 M NaCl, adjusted to pH 7.2 with drops of 1 M NaOH, referred to as "HEPES" [14]. The dehybridization solution was 0.5 M NaOH, with 3 M NaCl, referred to as "NaOH".

DNA biosensor oscillator frequency changes recorded during successive circulation of DNA solutions are presented in fig. 7. There is a first Δf_A = -1560 Hz frequency change during circulation of a 20 μg/mL DNA-disulfide NaCl solution attributed to chemical adsorption of the DNA-disulfide probe A on the gold surface of the quartz (1). Kinetics of the immobilization reaction were estimated by calculating Δt = t¾ - t¼, where t¾ and t¼ are respectively the ¾ and ¼ reaction time. DNA-disulfide adsorption Δt is equal to 6120 s. The next frequency shift is attributed to increase of viscosity and density between NaCl and HEPES solutions. There is no frequency shift during circulation of 20 μg/mL non-complementary DNA B and DNA C HEPES solutions indicating that there is no hybridization or non-specific adsorption of the non-complementary DNA strands B and C. There is a Δf_A = -1676 Hz frequency change during circulation of a 20 μg/mL complementary DNA A

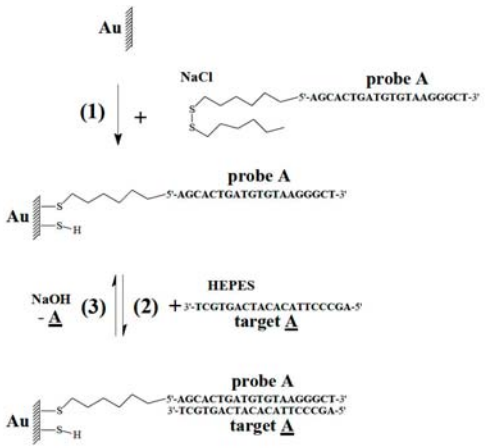

Fig.6. DNA-disulfide biosensor: immobilization of DNA-disulfide probe A (1), hybridization of a complementary DNA target A (2) and dehybridization of the DNA target A (3).

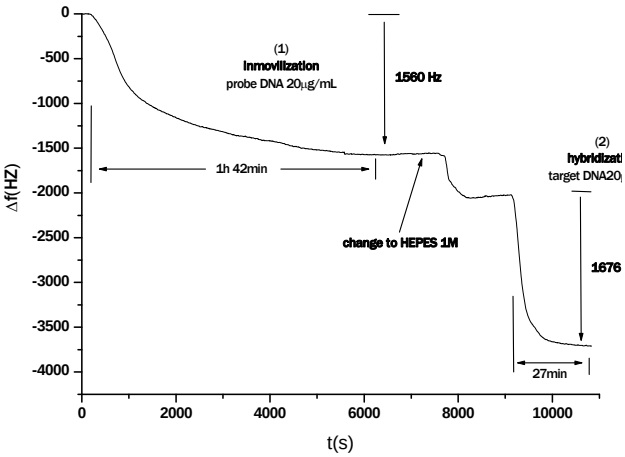

Fig.7. DNA-disulfide biosensor using the designed 50MHz QCM oscillator circuit: frequency changes during successive circulation of 20 µg/mL DNA-disulfide probe A NaCl solution (1), 20 µg/mL DNA target B HEPES solution, 20 µg/mL DNA target C HEPES solution, 20 µg/mL DNA target A HEPES solution (2)

solution in HEPES attributed to hybridization of the complementary DNA target A with the biosensor DNA probe A (step 2). The corresponding hybridization ratio η of hybridized DNA strands A, N_A vs. immobilized DNA-disulfide probes A, N_A is estimated to be 58%: $\eta = N_A/N_A = (\Delta f_A.M_A)/(\Delta f_A.M_A)$, where M_A = 6500 g/mol is the molecular weight of the DNA-disulfide probe and M_A = 12000 g/mol is the molecular weight of the DNA target A. The half-time hybridization reaction Δt calculated as indicated previously is equal to 1620s.

The DNA-QCM 50MHz oscillator biosensor designed is selective, as there is no frequency change of the QCM during circulation of the non complementary DNA B and C HEPES solutions. Also, this 50MHz DNA biosensor is high sensitive, because there was a -1671 Hz frequency change during circulation of a 20 µg/mL DNA target A HEPES solution, in front of -55 Hz that can be found using a DNA-QCM 27 MHz oscillator biosensor designed and studied in previous works [1] (see figure 8). Also probe A can be dehybridized by circulation of a NaOH solution and hybridized again with the complementary DNA target A.

Finally, a calibration of the DNA-QCM 50MHz oscillator biosensor with buffer solutions of different target DNA concentrations was carried out. In fig. 9 the obtained frequency curves are shown. It was founded that the designed 50MHz oscillator is able to detect DNA target A concentrations higher to 0.05µg/mL.

REFERENCES

[1] M. Lazerges, H. Perrot, N. Zeghib, E. Antoine, C. Compere, In situ QCM DNA-biosensor probe modification, *Sensors and Actuators B* 120, (1), 329-337, 2006

[2] L. Rodriguez-Pardo, J. Fariña, C. Gabrielli, H. Perrot and R. Brendel; "Sensitivity, noise, and resolution in QCM sensors in liquid media" *IEEE Sensors Journal*, 5 (6), 1251 – 1257, 2005

[3] V.E. Granstaff and S.J. Martin. Characterization of a thickness-shear mode quartz resonator with multiple nonpiezoelectric layers, *J. Appl. Phys*. 75 (3), (1994) 1319-1329.

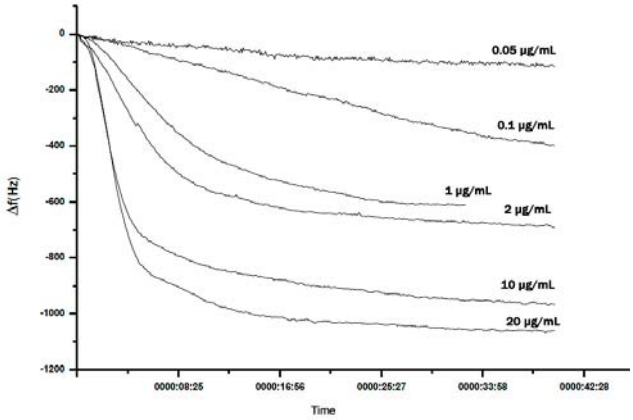

Fig.9. DNA-disulfide biosensor using the 50 MHz QCM oscillator circuit: frequency changes during the circulation of different concentrations of DNA target A HEPES solutions.

[4] Barnes, C. "Development of quartz crystal oscillators for under-liquid sensing"; *Sensors and Actuators A*, Vol. 29 pp. 59-69, 1991

[5] L. Rodríguez-Pardo, J. Fariña, C. Gabrielli, H. Perrot and R. Brendel, "Quartz crystal oscillator circuit for high resolution microgravimetric sensors in fluids", *Electronic Letters* 42 (18), 1065-1067, 2006.

[6] L. Rodríguez-Pardo, J. Fariña, C. Gabrielli, H. Perrot and R. Brendel, "Design considerations of Miller oscillators for high sensitivity QCM sensors in damping media", *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency control*, 54 (10), 1965-1976, 2007.

[7] L. Rodríguez-Pardo, J. Fariña, C. Gabrielli, H. Perrot and R. Brendel, "TSM-AW sensors based on Miller XCOs for microgravimetric measurements in liquid media", *IEEE Transactions on instrumentation and measurement*, 57 (10), 2309-2319, 2008.

[8] Matthys,R. "*Crystal Oscillators Circuits*" First edition, Ed. John Wiley & Sons, ISBN 0-471-87401-9, 1983

[9] H. Ehahoun, C. Gabrielli, M. Keddam, H. Perrot, Y. Cetre, L. Diguet, "EQCM corrosion sensor for solid metals and metal alloys. Application to the dissolution of 304 stainless steel", J. Electrochem. Soc., 148, 333-336, 2001.

[10] L. Rodríguez-Pardo, A. Cao-Paz, J. Fariña, A. Covelo, X.R. Novoa, C. Pérez "Monitoring of wáter uptake in anti-corrosion

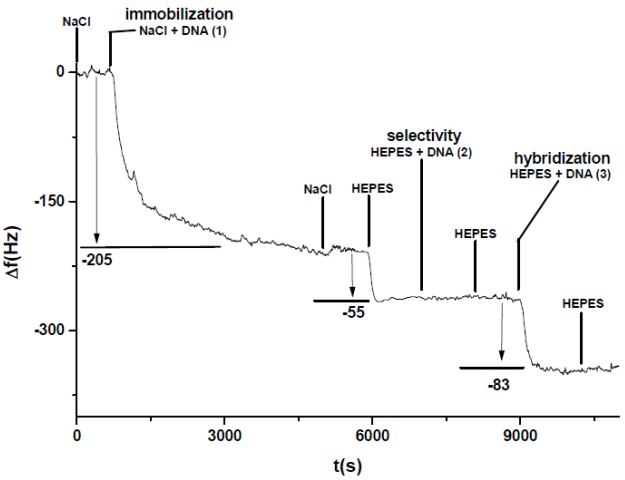

Fig.8. DNA-disulfide biosensor using a 27MHz QCM oscillator circuit: frequency changes during successive circulation of 20 µg/mL DNA-disulfide probe A NaCl solution (1), 20 µg/mL DNA target B HEPES solution, 20 µg/mL DNA target C HEPES solution (2), 20 µg/mL DNA target A HEPES solution (3)

organic coatings using a quartz crystal oscillator sensor", IECON 2008 – 34 Annual Conference on IEEE Industrial Electronics, 2008.
[11] Wide Bandwith Operational Transconductance Amplifier and Buffer, Texas Instruments, http://focus.ti.com/lit/ds/symlink/opa660.pdf
[12] D.W. Allan, "Statistics of Atomic Frequency Standars", proceedings of the IEEE Vol. 54, No.2, 1966
[13] G. Sauerbrey, Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mirkowägung, Z. Phys, 155 (1959) 206-222.
[14] C. X. Zhou, L. Q. Huang, S. F. Y. Li, Microgravimetric DNA sensor based on quartz crystal microbalance: comparison of oligonucleotide immobilization methods and the application in genetic diagnosis, Biosens. Bioelectron. 16 (2001) 85-95.