Quartz Crystal Microbalance for Medical Diagnostics

Matthew J. van der Werff[1], Yong J. Yuan[2] and W. L. Xu[1]

[1]Institute of Technology and Engineering, Massey University, Palmerston North, New Zealand

[2]Industrial Research Limited., Lower Hutt, New Zealand

Abstract

The research is to develop techniques to improve disease detection using a Quartz Crystal Microbalance (QCM) to induce and detect the Bond Rupture of the antibody–antigen bonds. In this paper a short introduction to QCM is presented and the techniques of how it is used to detect the bond rupture are reviewed. Issues involved in the detection such as frequency stability, viscosity, temperature control and others are discussed. Experimental setup is built where closed-loop temperature control is implemented using PID, and Network Analyzer is used to provide accurate resonant frequency measurements in conjunction with Matlab to automate measurements. Preliminary meaningful results with in liquid measurements of QCM's are given in the paper.

Keywords: Quartz Crystal Microbalance, QCM, Bond Rupture, Biosignal Processing, Antibody-Antigen.

1 Introduction

In the medical world the precise identification of a disease can take longer than it is safe to wait to start treatment which is why doctors are often forced to treat disease from initial symptoms. Diagnostic tests are also important in the pharmaceutical industry to test how effective a cure is on a disease. Traditional antigen identification has been performed using direct culture techniques that are very time consuming and may take hours or days to give a result. Many differing types of methods for the identification of antigens exist including: fluorescence labeling, radioactive labeling, optical scanning, and oligonucleotide-based assays, to name a few all with there individual advantages and disadvantages, but most trading of sensitivity for speed. Other methods include biosensors such as Surface Plasmon Resonance (SPR), Potentiometric biosensors, and Quartz Crystal Microbalance (QCM) [1]. All of the above can detect surface binding of antibody-antigen though they also have problems related to unknown antigen bonding to the antibody but, with a much weaker bonding force (non-specific bonding). To get around the non-specific bonding problems a method was proposed by Dultsev [2] that measures the bond strengths of the attached antigen by applying an increasing voltage to a QCM while also measuring the loss of mass to the QCM in so creating a spectrum of the bond forces. This precise identification of antigen via their bond strength and may also allow different antibodies to be placed on the surface of a sensor for a multiple antigen sensor.

1.1 Quartz Crystal Microbalance (QCM)

Sauerbrey [3] demonstrated in 1959 that the resonate frequency change was proportional to the deposited mass on the quartz crystal and hence the Quartz Crystal Microbalance (QCM) was born. Eq. (1) was proposed to find the amount that the frequency changes with mass accumulation being dependent on harmonic frequency (f_0), the area over which the mass is spread (A), and material properties of the quartz (c_{66} and ρ_q). This was found to be as accurate enough to measure around nano-gram changes of mass or measure mass changes as small as a fraction of a monolayer.

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\bar{c}_{66}\rho_q}}\Delta m = -S_f \Delta m \qquad (1)$$

Konig [4] used the mass change to detect biological particles attaching to the surface such as in the example in figure 1. The steps to performing an antigen using its corresponding antibody are as follows; 1) Attach the Self Assembling Monolayer (SAM) to the surface of the QCM forming a Sulphide-Gold bond, 2) Attach the antibody to the SAM via a Nitrogen-Carbon bond, 3) Detect the antigen via change in frequency due to mass addition. In reality there are many other factors that cause frequency change so to measure mass change accurately the other contributing factors must be held constant. These factors include: temperature, humidity, liquid viscosity and density, flow rate, and micro bubbles, which all will be addressed in section 2.1.

The method proposed by Konig [4] has been used as a reasonably effective biosensor for detection of

Listeria monocytogenes a common food pathogen by Vaughan [5]. The problem with this method is that non-specific antigen-antibody bonds can not be told apart from the specific antigen-antibody bonds, which means great care must be taken to choose antibodies that are not susceptible to this and also to minimize other antigens when processing the sample solution, where as Bond Rupture should work with less specific antibodies.
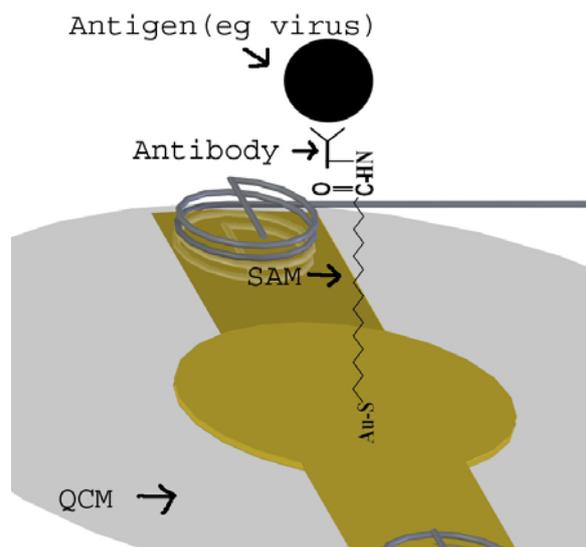

Figure 1: Rendered 10MHz Quartz Crystal Microbalance (QCM) with my rendition of the Self Assembling Monolayer (SAM), Antibody, and Antigen.

1.2   Bond Rupture

To get around the non-specific bonding problem Dultsev [2] proposed to measure the bond strength of all the attached antigen by applying an increasing voltage to a QCM to induce rupture of the antibody-antigen bonds while simultaneously measuring the loss of mass or noise at the third harmonic from Bond Rupture. This enables the classification of specific and non-specific antigen-antibody bonds. Cooper [6] applied Eq.(2) to calculating the force between the antibody and antigen, with the force ultimately dependant on the frequency of the crystal (f), the mass of the antigen (m), and the driving amplitude (A). The driving amplitude was derived by Borovsky [7] in Eq. (3) and depends on the Quality factor (Q) and the driving voltage (V). Although this is maybe a good approximation, Borovsky only did measurements up to around 1V. Therefore, this may not hold true for voltages we need (around 7V) so further measurements need to be completed to determine the viability of Eq.(3).

$$F = \tfrac{2}{7} mA(2\pi f)^2 \qquad\qquad (2)$$

$$A = 1.4QV \qquad\qquad (3)$$

The force range required to rupture a typical antigen-antibody bond has been determined by Weisel [8] to be around 100pN +/- 50pN for single bonds although multiple bonds can be formed between the antigen and antibody. The force as calculated by Cooper [6] which the QCM can apply to a single particle with a QCM frequency of 14.7MHz, voltage of 7V and a Q of 1500 gives an acceleration of 3.4×10^7 ms^{-2}. Hence, assuming an antigen weight of 80fg the force on the antigen is about 3nN. So for a rupture at 7V there could be around 30 antibody bonds to the antigen. This still does not take into account the multiple bonds changing the dynamics of the system from the simple one bond antigen-antibody model or the effects that liquid could have.

The method used by Dultsev [2, 9], Cooper [6, 10], and Kulin [11] to detect bond rupture was to oscillate the QCM at an increasing voltage around the first harmonic using a signal generator and then using a Lock-in Amplifier tuned to the third harmonic recording the output in relation to driving voltage (refer to figure 4). This measures the noise given off by the antigen-antibody bonds breaking as this produces wideband noise which will only be present at the frequencies that the QCM is sensitive at which is the odd harmonics. The reason the third harmonic was used in Dultsev's experiment was because the first harmonic is used to drive the QCM and so would have too much signal on it already.

2   Design Considerations

The first aim was to stabilise the QCM's Environment factors that affect the frequency then building equipment to take into account these factors. The next aim was to decide on methods to measure the frequency change and also monitor the noise at the third harmonic.

2.1   Frequency Stability

When measuring the attachment of mass in such small scales on the QCM it is important to have a high frequency stability and low frequency noise to increase mass sensitivity. Therefore the first aspect of this project was to isolate any influential environment factors that cause frequency drift such as: temperature, humidity, liquid viscosity and density, flow rate, and micro bubbles.

The temperature change verses frequency change is represented by a Quadratic Polynomial as found by Bouzidi [12] with the crystal being most stable at the minimum of the curve which in Bouzidi's case is around 80°C (figure 2). This high temperature is not practical for the biological fluids that we are working with so the temperature must be kept very stable. Accurate temperature control was implemented using a Peltier element which can heat or cool depending on the direction of the current so when using a

differential power supply the temperature can be controlled within 0.05°C (see section 2.2).

Humidity causes a change in frequency on the QCM because of micro droplets forming on the air side of the crystal causing a mass change. Stable temperature helps keep the humidity constant so no change in frequency but ideally to get rid of the humidity problem nitrogen can be run over the air side.
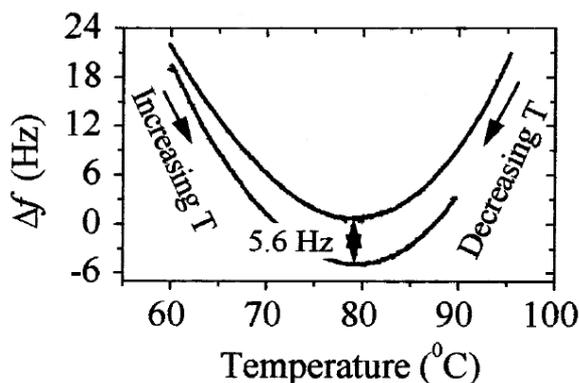

Figure 2: Resonance frequency vs quartz temperature for warming and cooling cycles at 0.1 °C/min [12].

Liquid viscosity and density is easy to overcome by using constant liquid such as ethanol or water and mixing small amounts of the other chemicals, but when testing biological fluids such as within blood samples this is not quite as easy as it sounds and will need to be looked at further down the track when this stage has been reached.

Flow rate is also a contributing factor seemingly to increasing frequency noise as well as frequency drift as Sota [13] found changes from noise of ±0.15Hz and drift of 12Hz/h for 0 μL/min to around noise of ±4Hz and drift of ± 60 Hz/h for 100 μL/min. Careful design of the flow cell and choice of crystal can increase the characteristics to such as the rectangular Quartz resonator also mentioned in Sota [13] although further research must be done into this to decide whether this resonator is appropriate for Bond Rupture detection.

Micro bubbles' forming on the crystal from the supply of liquid also can cause frequency jumps ranging from about ±100Hz to ±1.5kHz. Micro Bubbles are introduced from a pump which forces bubbles though the system, a better solution would be to use a vacuum pump so that the air will be removed first. To see examples of the jumps caused by micro-bubbles see figure 7.

There are also other factors which can just be associated general degrading of the crystal such as frequency drift and the background noise, though this can also be caused by an inadequately cleaned QCM surface which can lead to mass loss/addition that just adds to the drift and noise.

2.2 Temperature Control Design

To keep the temperature constant a Peltier thermo-electric cooler/heater was used with a switched mode PID temperature control chip supplied by maxim (MAX1978) and a high accuracy calibrated thermistor to control our temperature within at least ±0.05°C. The controller can control accurately down to 0.001°C but noise in the potentiometer and thermistor make are much higher so to get higher temperature control better components must be used. The temperature at this stage is controlled by a high precision ten turn potentiometer which the MAX1978 compares with the thermistor value and is controlled by a PID controller that is implemented in hardware (Resistor and capacitor choice). It is planned to use a DAC to set the temperature and use an ADC to read the values to our system so that we can set and monitor the heat in software.

To transfer the heat to the liquid a primitive heat exchange system is used, as shown in figure 3. Because of our relatively low flow rate at this stage we can just run the pipes past the Peltier device using an aluminium plate with 4 holes just big enough to allow the biological grade pipe to be fed thru also with a hole drilled halfway thru for thermistor placement.
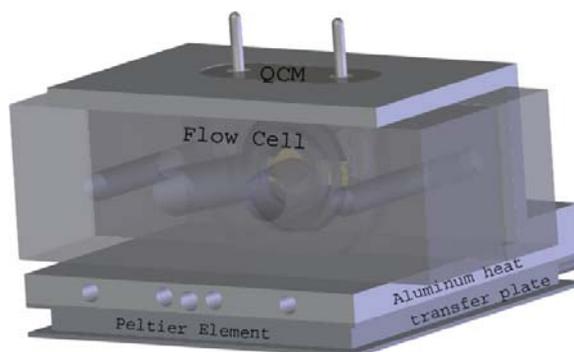

Figure 3: The Flow Cell, heat transfer plate, QCM and Peltier element.

The setup has managed to stabilise the frequency fluctuations with overnight tests showing only a steady rise which will be addressed in section 3.

2.3 QCM Analysis

There are many different methods to analyze QCM frequency change including; Oscillator circuits, impulse excitation, and impedance or phase analysis. These methods have their advantages and disadvantages for straight frequency analysis, but we also need to analyse the noise at an odd harmonic (other than the driving harmonic) for bond rupture events as well as being able to increase the output voltage and handle reading a variable input voltage. A method is described in Dultsev [9] using a sine wave generator to drive the QCM and a lock-in amplifier to measure the bond rupture (figure 4) but this is an expensive and bulky solution that also lacks

variability in the analysis setup as well as no way of monitoring the resonant frequency.

To improve on Dultsev's method we will be creating digital signal processing equipment as this should give us advantages such as: many different methods can be tried on the same board, digital filters can give measurable errors, we can abstract the problem e.g. a software problem rather than hardware, better automation is possible, real time processing is possible, and the price can be dropped.
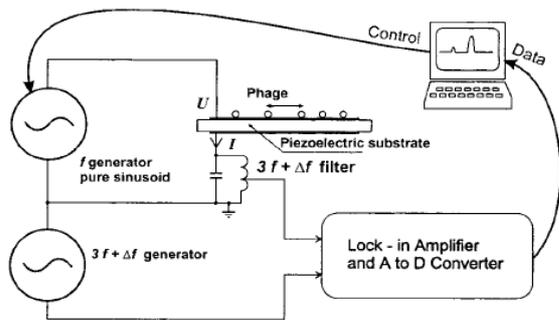

Figure 4: Bond rupture method by Dultsev [9] including variable amplitude sine wave generator and Lock-in Amplifier monitoring noise at the third harmonic for Bond Rupture events.

3   Experiments

One of the first objectives of this project was to stabilize the QCM's frequency and noise and determine the factors that affect its stability. To gather accurate measurements from the QCM an Agilent 8712ET Network Analyzer was used to provide accurate resonant frequency measurements using the maximum impedance measurement in conjunction with Matlab to automate measurements as seen in figure 5. A Matlab GUI (figure 6) was created to process and record the data from the Network Analyzer, and send commands to the Network Analyzer. The steps the Matlab program runs though when measuring are: 1) First a TCP/IP port connection is establish to the network analyzer to send the initial SCPI commands to setup the initial frequency range and set the measurement type to impedance. 2) Once waiting for Analyzer to run a scan the data is downloaded as a CSV file via ftp. 3) The data is de-noised using the wavelet toolbox in Matlab. 4) The maximum impedance point is found or if zoomed in close enough a 2nd order polynomial fit is used to find the maximum impedance point. 5) Zoom in to data unless at maximum zoom. 6) Record maximum impedance frequency and draw graph. 7) Go back to step 2. This gives around 0.5Hz frequency resolution in air and about 2Hz resolution in liquid.
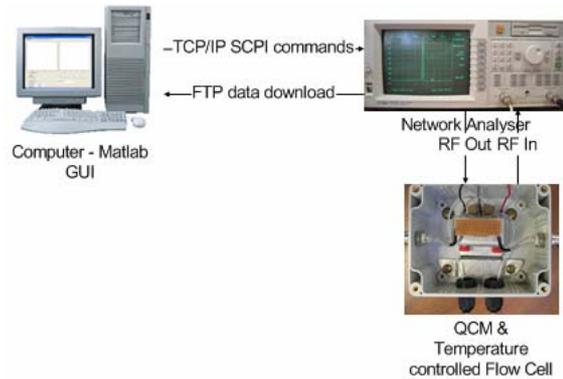

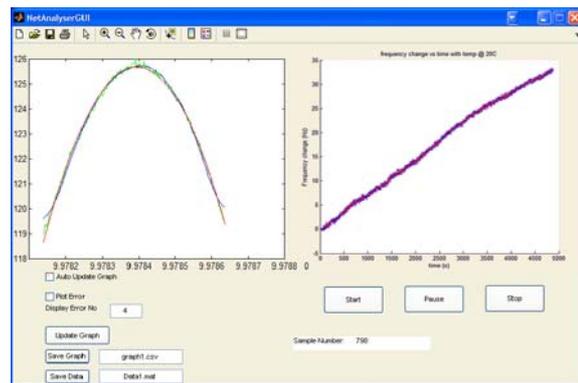**Figure 5:** Current experimental setup

Figure 6: Matlab GUI for recording frequency change (left side is polynomial fitting of impedance from network analyser to find harmonic frequency and right side is recording frequency change verses time).

The main frequency change factor was the temperature as shown in figure 6 as if the temperature of the water is changed from 45°C to 3°C that the frequency change is around 1000Hz. The next most important problem was micro bubbles forming on the surface of the QCM causing large jumps in frequency as also shown in figure 7 which can easily be compensated in software by adding/subtracting the difference onto the zero point reference frequency each time a jump of over 50Hz is detected. This is only a temporary measure until a vacuum pump system is implemented as bond rupture could also produce similar results.

We also found that keeping temperature constant at 20°C and flow rate constant in water that there it a constant drift of around ~30Hz/h (figure 8), which maybe attributed to QCM drift, mass loss from flow, or network analyzer oscillator drift. Due to the constant frequency change it is probable that it is due to a mass loss which if correct would be fixed by using proper QCM cleaning techniques. Longer time periods are needed to confirm that the frequency change slope is constant and further experimentation to see the effect of flow rate on this.
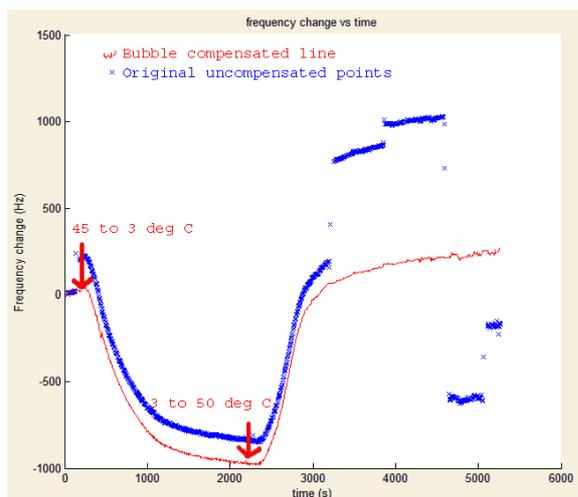
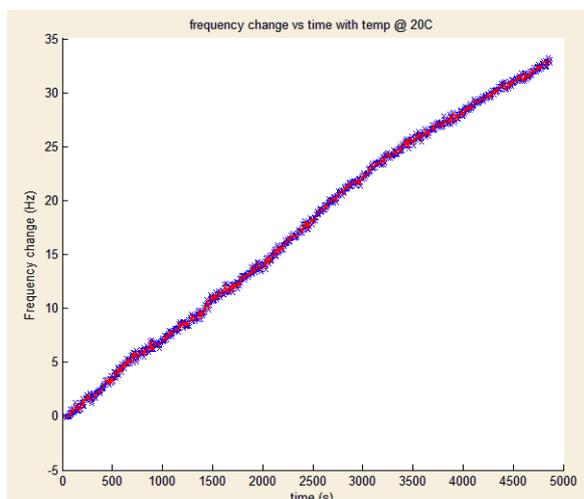**Figure 7:** Captured frequency change from 45°C to 3°C then up to 50°C in water.

Figure 8: Captured frequency change over 1hr 20min at 20°C in water.

4   Conclusion

This paper reviewed the state of the art of QCM to Bond Rupture detection with applications into medical diagnosis. Initial experimental system has been set up where the Network Analyzer was used to provide accurate resonant frequency measurements in conjunction with Matlab to automate measurements and closed-loop PID temperature control is implemented. The preliminary results have proved that our proposed methodology is sound for QCM frequency change detection but further work must be done to implement bond rupture detection.

5   Acknowledgements

6   References

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