[1] Dr. P Sangeetha
[2]Dr. Ankayarkanni B

Design and Development of Piezoelectric Biosensor for Enhanced Pathogen Detection

Journal of Electrical Systems

Abstract: - A biosensor is a device which transforms a biological response into an electrical signal. It uses a bioreceptor which consists of a biologically sensitive material to detect biological species. This bioreceptor acts as the recognition element and is attached to a transducer. The transducer will convert the response to an electrical signal. This signal is proportional to the concentration of the target biological substance. The biologically sensitive material can include enzyme, molecules, lectins, receptors, antibodies etc. This biosensor uses the reaction between antibody and antigen hence can also be called as immunosensors. The biosensor reacts to this antigen antibody binding. The transducer converts this product into a conventional electrical signal. The electrical signal can be processed with amplification and further displayed.

Keywords: Antibody, antigen, biosensor, bioreceptor, quartz crystal.

1. INTRODUCTION

The importance of this research is to detect disease causing pathogens using biosensors and analyze the read-out technique. Any infectious biological agent like a virus, bacterium, or other microorganism which is responsible for causing a disease is called a pathogen. In this research, detection of Tb pathogens using biosensors is analyzed. World health organization (WHO) has declared TB as a global public health emergency as it causes illness among millions of people each year. The bacillus Mycobacterium tuberculosis is responsible for this infectious disease which can affect the lungs and other parts of our body. The diagnosis of TB in the early stages is very important. This biosensor can be used to detect the presence of TB.

2. PIEZOELECTRIC EFFECT

When a mechanical force is applied, the crystal dimensions change resulting in accumulation of electric charges on the surface in which force is applied. The applied force is proportional to the charge accumulated. This effect is called the piezoelectric effect. This effect is used to measure parameters like acceleration, pressure, force, strain and vibration. The accumulated charge and the applied force are related by equation (1),

$$Q = cF \qquad (1)$$

Where Q is the accumulated charge, F represents the applied force and c is the piezoelectric constant. The piezoelectric effect seen by applying a force (Fig. 1). The orientation and mode of operation decides the value of 'c'. The way the sensors are cut from the crystal determines the orientation. There are four types of cut like shear cut, longitudinal cut, polystable cut and transverse cut. The method by which stress is applied determines the mode of operation. Bend mode, shear mode and compression mode are the various modes of operation. In operating mode piezoelectric crystal sensor behaves like a parallel plate capacitor.

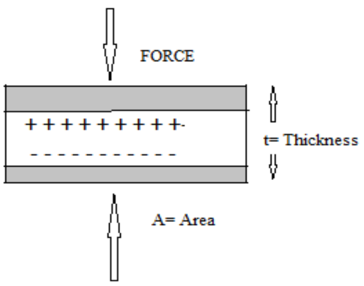

Fig. 1 Piezoelectric effect

[1] *Department of Electronics and Communication Engineering, Rajiv Gandhi college of Engineering, Research and Technology, Chandrapur, Maharashtra, India
[2] Department of Computer Science and Engineering, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India
[1] sangeethakalaiselvan1@gmail.com, [2] ankayarkanni.s@gmail.com

3. PIEZOELECTRIC MATERIALS

A. Materials

Materials that are permanently polarized and crystalline in nature are called piezoelectric materials. A piezoelectric material is not symmetric in all directions. The symmetrical nature of the material will not show electric polarization. The symmetric material shows no electric polarization by the application of force.

B. Quartz crystal as piezoelectric material

The force or pressure, when exerted on certain crystals, the molecules get polarized and produce a charge on the surface of crystal. This charge is measured as a voltage. The voltage generated from a crystal can be calculated from equation (2).

$$V = S_v * t * P \tag{2}$$

'V' is the generated piezoelectric voltage measured in Volts.Voltage sensitivity of the material measured in V-m / Newton is denoted by 'S_v'.

Voltage sensitivity values are given by the manufacturer. Voltage sensitivity values depend on the material and its geometry. Pressure 'P' is measured in N/m^2 and 't' denotes the thickness of the piezoelectric material. Rochelle salt, barium titanate, zirconate titanate, ammonium dihydrogen phosphate and quartz are some of the piezoelectric materials. Piezoelectric effect is an important property of quartz crystal. High frequency stability and accuracy can be obtained by using the quartz material as the crystal. In applications where frequency stability is important, quartz crystal play an important role. Quartz material is easily available in nature and less expensive too. Pyramid ends with hexagonal prism is the natural shape of the crystal. To extract the crystal for use, a rectangular slab is cut from the natural crystal. AT cut, X-cut, Y-cut, XY-cut are the various methods and types to cut the crystal, every cut has its own piezoelectric properties. Piezoelectric properties like resonant frequency, temperature coefficient, etc is determined by the type of cut. The rectangular slab is sandwiched between two metal plates and encapsulated. By applying voltage to the metal plates, crystal starts vibrating. The crystal vibrations reach a high value at a particular frequency called the resonant frequency of the crystal. The resonant frequency range of the crystal from 15 KHz to MHz is available.Fig.2 shows the mounted quartz crystal.

C. Quartz Biosensor

The quartz crystal integrated with signal processing system works as a biosensor. The sensor shows good sensitivity and is compact in size. The sensor can be extended for point of care and home care applications as it can be designed as a low-cost device.

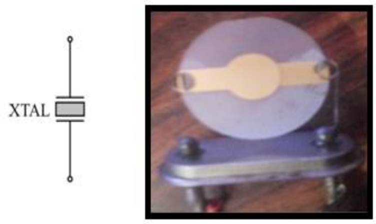

Fig. 2 Quartz crystal

D. Quartz Crystal Oscillator Circuits

In feedback amplifiers if the feedback is in such a way that increases the input and is in phase with the input then the feedback is positive feedback or direct feedback. The positive feedback gives feedback amplifier a close loop gain A'_v which is more than open loop gain Av. This leads to an oscillatory circuit which provides an output signal of a required frequency.

Clapp crystal oscillator, Colpitts crystal oscillator, crystal controlled tuning oscillator, pierce crystal oscillator are the commonly used crystal oscillator in use. The transistor crystal oscillator is shown in Figure 3. The collector to base feedback path consists of the crystal in series connection. Stable DC bias is provided with a voltage divider circuit by resistors R1, and R2. C is the coupling capacitor having less impedance at the operating frequency and aids in blocking DC between collector and base.

The A.C bypass of the resistor in the emitter terminal is provided by the capacitor CE.The DC bias is provided by the radio frequency choke (RFC) and helps in decoupling the AC signal on the power lines from changing the output signal. The frequency of oscillation of the circuit is established by a series resonant frequency fs which is given by Equation 3

$$f_s = \frac{1}{2\pi\sqrt{LC_1}} \quad (3)$$

Factors like supply voltage changes, transistor parameter variation will not alter the circuit resonant frequency as the frequency stability is established by the crystal.

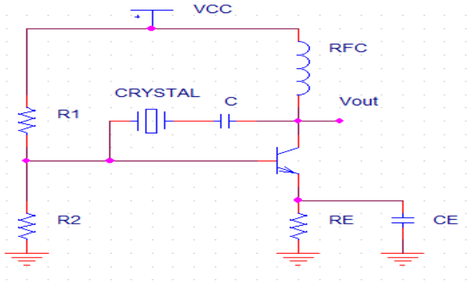

Fig.3 Quartz crystal oscillator

The Quartz Crystal oscillator shows piezoelectric readout and highly sensitive to detect changes in mass. Fig.3 shows a quartz crystal oscillator. Single layer of molecules coated on the surface can also be detected. This can be a low-cost sensor showing good accuracy. When mass is added to the surface, the resonant frequency changes. The effect of mass changes on the surface of the crystal [1] is given by Equation 4.

$$\frac{\text{Change in mass}}{\text{Original mass}} = \frac{\text{Change in frequency}}{\text{Original frequency}} \quad (4)$$

E. Quartz Crystal Biosensor for disease Detection

Prevention of diseases and effective treatment of those diseases is possible only by proper diagnosis and early treatment. Rapid development of piezoelectric biosensors and their analytical potentials are used for deployment in various biosensing applications [2],[3]. Continuous measurements of proteins like antibodies are possible with the help of quartz crystal biosensor [4], [5],[6]. Mass sensitivity in the order of picogram using quartz sensor is observed. Sensitive quartz biosensors for detecting M13-Phages in liquids with sensitivity in the order of nanogram have been reported [7]. Immobilized antibody on the sensor platform binds with the analyte causing a change in the resonant frequency of the sensor. This change in frequency can be recorded using a high-resolution frequency counter. This method is used for immunological measurements [8]. This biosensor consists of a recognition element like an antibody along with a transducer which convert the interaction between the analyte and the recognition element into electrical signals. These biosensors do not require reporter labels to analyze the molecular activity [9]. Quartz is a piezoelectric material, it deforms if an electric field is applied to it and, conversely, generates an electric field in response to mechanical stress. Piezoelectric property of the crystal is used for biosensing. Mechanical oscillations of the crystal are produced when a voltage is given to the electrodes. The oscillator is able to produce stable oscillations due to the high Q factor of the crystal. The frequency of oscillation changes in proportion to the amount of mass when a mass is adsorbed or placed onto the quartz crystal surface. These devices can be used as biosensors intended to measure mass changes by coating the crystal with an antibody which is selective towards the species of antigen. Detection of pathogens is done by coating the crystal with specific antibody. Fig.4 shows the structure of a biosensor based on a quartz crystal, oscillator and counter. The quartz crystal acts as a signal transducer, converting mass changes due to the antigen antibody binding process into frequency changes. The equation describing the linear relationship of frequency change (Δf) and mass loading (Δm) on a Quartz crystal is given [1] in Equation 5.

Δf = -2.3 *10-6fo2 Δm/A (5)

Where Δm is the change in deposited mass, coated area is represented by A, the frequency of the quartz crystal is denoted by fo and Δf is the change in frequency of the crystal. The antibodies that are specific to antigen is to be coated on the Quartz crystal. When the blood sample is placed on the crystal, if the patient is affected by a disease, antigen will be present in the blood sample. Antigen antibody binding will take place, causing a change in mass. This change in mass causes a change in resonant frequency. This change in mass is proportional to frequency change indicating the antigen antibody binding.

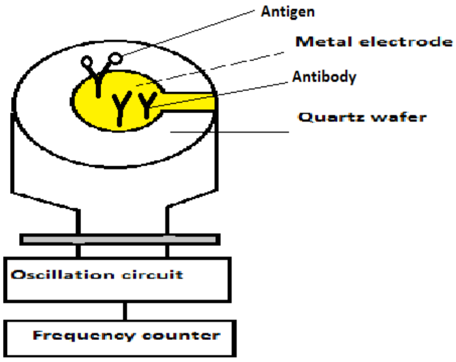

Fig.4 Illustration of Quartz crystal biosensor with gold coated quartz as the sensing surface

4. Results and Discussion

The proposed circuit can monitor a small frequency variation of even 1Hz and signal of low frequency in the range of 1Hz can be obtained by mixing the signal from the crystal oscillator sensor and the reference signal from a reference oscillator. A small variation can also be measured by this mixing and this signal can be further processed for display. 10 MHz crystal has an 8mm diameter and a thickness of 0.1 mm. Fig. 5 shows the block diagram of biosensor with reference oscillator.

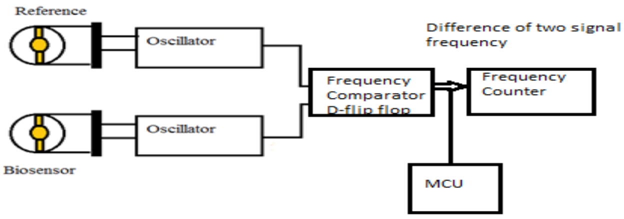

Fig 5 Block diagram of biosensor with reference oscillator

One side of the crystal is kept open to the sample which can detect changes as small as 1 Hz. AT-cut crystal working in shear mode is used. The crystal used is HC49/U model and connected as 10 Mhz crystal in the circuit. The crystal of this frequency is widely available. The mass added in the range of 2 to 3 nanograms will produce a change of 1Hz.The crystal that produce frequencies higher than 10 MHz are weak and delicate. Sensitivity is less for lower frequency crystal.10 MHz is the best frequency for biosensing and mass change detection.74LS04 IC can be used as oscillator where the amplifier part is executed by two gates which are the amplifier digital invertors. The crystal has its own resonant frequency. LC filter is used to set the fundamental frequency of the crystal oscillator. The values of L and C give the resonant frequency as 10 MHz Fig 5 illustrates the diagram of Quartz crystal biosensor with reference and sensing circuit. The reference oscillator is same as sensor oscillator only difference is that the crystal is not open for mass loading. Fig 6 shows the circuit of Quartz crystal biosensor with reference and sensing circuit. The oscillator with the reference crystal will produce signals with a frequency which remains static as the crystal is unloaded. The difference between the frequencies of the reference and sensor oscillator can be obtained by using a comparator. This frequency comparison is done by a D-type flip-flop. The two outputs from sensor oscillator and reference oscillator should be given to the data input and to the clock input of the D-type flip-flop. The flip-flop at the output will give a square wave whose frequency is the difference of two signal frequency. The frequency of the signal at the output of the comparator was measured using a frequency counter and with a microcontroller. The Voltage divider circuit at the output of the comparator gives low level voltage of 0.5 volts that can drive analogue inputs. Fig 6 shows the simulation output of the Quartz crystal biosensor circuit. Fig 7 shows the simulation of Quartz crystal biosensor output waveforms. Fig 8 shows the simulation of Quartz crystal biosensor circuit where the output from both the oscillators is given to the flip flop. Fig. 9 shows circuit diagram simulation of the Quartz crystal biosensor with reference oscillator.

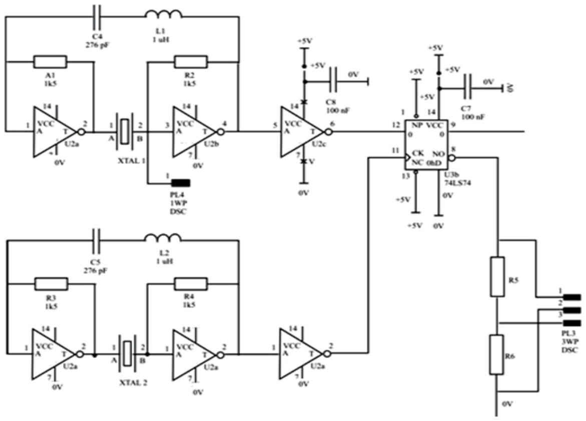

Fig 6 Circuit diagram of Quartz crystal biosensor with reference and sensing circuit

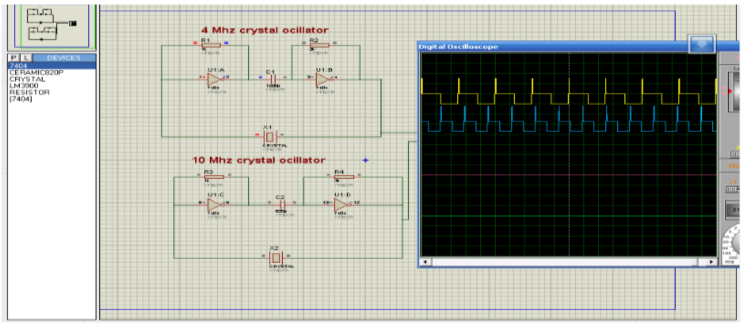

Fig 7 Simulation of Quartz crystal biosensor circuit

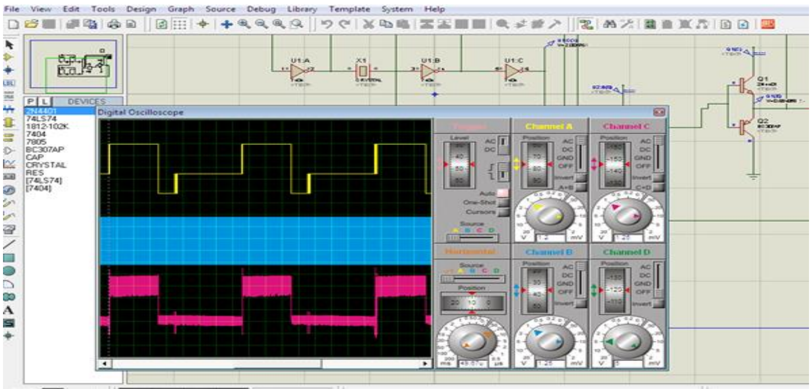

Fig. 8 Simulation showing the waveforms of Quartz crystal biosensor circuit.

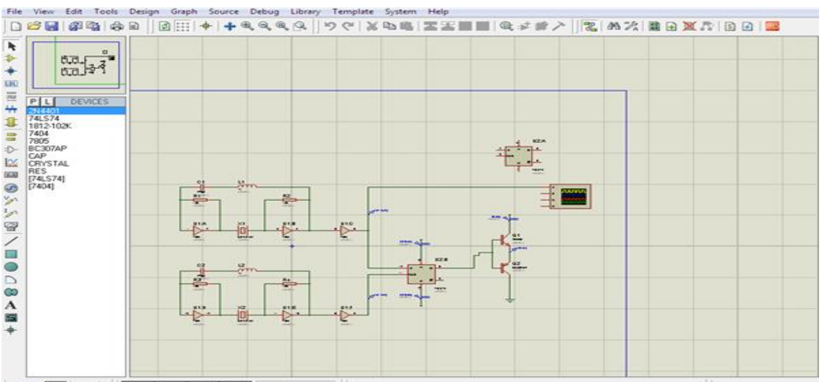

Fig. 9 Circuit diagram simulation of the Quartz crystal biosensor with reference oscillator

5. Conclusion

Biosensing systems like these can be connected to computer for online inspection of bio affinity reaction .The demand for low cost, point of care biosensors for disease causing pathogen detection initiated and encouraged this research on biosensors. Early detection of pathogens is important for medical treatment. Piezoelectric readout with quartz crystal biosensor is designed. It is noted that crystal-based biosensor can provide better biosensing

References

[1] Sauerbrey, G. (1959), "Use of quartz vibrator for weighing thin films on a microbalance", Z. Phys., Vol. 155, pp. 206-210.

[2] Vaughan, R.D. and Guilbault, G.G. (2007), "Piezoelectric Immunosensors", In: Steinem C, Janshoff A, eds. Piezoelectric Sensors, Springer Berlin Heidelberg, Vol. 5, pp. 237-280.

[3] Ermolaeva T.N., Kalmykova, E.N. and Shashkanova, O Yu. (2008), "Piezoquartz Biosensors for the Analysis of Environmental Objects, Foodstuff and for Clinical Diagnostic", Rus. J. General Chem., Vol.78, No.12, pp. 2430-2444

[4] Sadik, A. and Cheung, M.-C. (2001), "Monitoring the specific adsorption of proteins using the electrochemical quartz crystal microbalance electrodes", Talanta, Vol.55, No.5, pp. 929-941.

[5] Storri, S., Santoni, T., Minunni, M. and Mascini, M. (1998), "Surface modifications for the development of piezoimmunosensors", Biosens. Bioelectron., Vol.13, No. 3-4, pp. 347-357

[6] Sakti, S.P., Hauptmann, P., Zimmermann, B., Buhling, F. and Ansorge, S. (2001), "Disposable HSA QCM-immunosensor for practical measurement in liquid", Sens. Actuators, Vol.78, No.1-3, pp. 257-262.

[7] Uttenthaler, E. et al., (2001), "Ultra sensitive quartz crystal microbalance sensors for detection of M13-Phages in liquids", Biosensors and Bioelectron., Vol. 16, pp. 735-743.

[8] Kosslinger, C., Drost, S., Aberl, F., Wolf, H., Koch, S. and Woias, P. (1992), "A quartz crystal biosensor for measurement in liquids", Biosens. Bioloectron., Vol.7, pp. 397-404.

[9] Matthew A. Cooper and Victoria T. Singleton (2007), "A survey of the 2001 to 2005 quartz crystal microbalance biosensor literature: applications of acoustic physics to the analysis of biomolecular interactions", Journal of Molecular Recognition, Vol.20, pp.154-184 Published online in Wiley InterScience (www.interscience.wiley.com) DOI:10.1002/jmr.826