

## Biosensor based on piezoelectric crystal probe

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### ABSTRACT

A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component. It consists of many parts such as the Sensitive Biological Element, the Probe and Electronics. Biosensor has many advantages such as minimum sample pretreatment, fast response time, simple and low-cost instrumentation and high sample output. Biosensor is beneficial due to high selectivity and sensitivity of a one known biologically active material. The resonant frequency of an oscillating piezoelectric crystal can be affected by varying mass at the crystal surface. Piezoelectric immunosensor is capable of measure a small change in mass. The empirical equation for AT-cut quartz crystal vibrating in thickness shear mode that describes relation between mass of thin metal films deposited on quartz crystals and corresponding change in resonant frequency of piezoelectric crystal is given by:

$$dF = -2.3 \times 10^6 F^2 dM / A$$

Where  $dF$  is the frequency change in oscillating crystal in Hertz,  $F$  is frequency of piezoelectric quartz crystal,  $dM$  is mass variation and  $A$  is the area of electrode. The construction of an antibody-based piezoelectric sensor capable of detecting mycobacterial antigen in diluted cultures of attenuated *M. tuberculosis* is discussed in this work.

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### INTRODUCTION

Detector is the component of biosensor. The function of a transducer is to convert high frequency voltage into ultrasonic wave and vice-versa. In normal probe; the waves are normal to be emitting surface i.e. in contact testing these waves enter the test surface at right angle. In angle beam probe; a plastic wedge is inserted between emitting surface and piezo-electric plate. When testing, ultrasonic wave is refracted at the interface between plastic wedge and test surface and penetrates the specimen at an angle. The detector, which plays the role of the transducer, translates the physical or chemical change by recognizing the analyte and relaying it through an electrical signal. The biological-sensing element selectively recognizes a particular biological molecule through a reaction, specific absorption, or other physical or chemical process, and the probe converts the result of this recognition into a usable signal, which can be quantified. Common transduction systems are optical, electro-optical, or electrochemical; this variety offers many opportunities to tailor biosensors for specific applications. The glucose concentration in a blood sample can be measured directly by a biosensor, which is made specifically for glucose measurement by simply dipping the sensor into the sample.

A biosensor is an analytical tool consisting of biologically active material used in close conjunction with a device that will convert a biochemical signal into a quantifiable electrical signal. Biosensors have many advantages, such as simple and low-cost instrumentation, fast response times, minimum sample pretreatment, and high sample throughput. Although biosensors are beginning to move toward field testing and commercialization in the United States, Europe, and Japan, relatively few have been commercialized. Increased research in this area demands the development of novel materials, new and better analytical techniques, and new and improved biosensors.[1- 4]

The objective of the research described here is to use biosensor technology to develop a rapid method for the diagnosis of tuberculosis and other infections caused by mycobacteria. The work encompassed here describes the construction of antibody-based piezoelectric crystals capable of detecting mycobacterial antigens in diluted cultures of attenuated *M. tuberculosis* in an immunologically specific manner. The antigen were detected in either liquid or vapor phase.

## 2. PIEZOELECTRIC BIOSENSOR

Piezoelectric sensors utilize crystals which undergo an elastic deformation when an electrical potential is applied to them. The applied alternating potential produces a standing wave in the crystal at a characteristic frequency. This characteristic frequency is highly dependent on the elastic properties of the crystal. If a crystal is coated with a biological recognition element the binding of a (large) target analyte to a receptor will produce a change in the resonance frequency, giving a binding signal. In a mode that uses surface waves (SAW), the sensitivity is greatly increased. This is a special application of the Quartz crystal microbalance in biosensor. The piezoelectric generator is the method of transforming high frequency electric oscillations into mechanical oscillations. It is most frequently used at the present day for producing ultrasonic and it allows us to attain the highest frequencies now possible. Two classes of bio-recognition processes-bio-affinity recognition and bio-metabolic recognition, offer different methods of detection. Both processes involve the binding of a chemical species with another, which has a complementary structure. This is referred to as shape-specific binding. In bio-affinity recognition, the binding is very strong, and the transducer detects the presence of the bound receptor-analyte pair. The most common types of processes are receptor-ligand and antibody-antigen binding. In bio-metabolic recognition, the analyte and other co-reactants are chemically altered to form the product molecules. The biomaterials that can be recognized by the bio-recognition elements are as varied as the different reactions that occur in biological systems. **Table 1** presents the lists a number of common analytes that could prove attractive for developing biosensors of appropriate specificity and sensitivity. Almost all types of biological reactions, (chemical or affinity), can be exploited for biosensors. The concept of shape-specific recognition is commonly used to explain the high sensitivity and selectivity of biological molecules, especially antigen-antibody systems. The analyte molecule has a complementary structure to the antibody, and the bound pair is in a lower energy state than the two separate molecules. This binding is very difficult to break.

Table 1- Selected Biosensor Substances

Sl.No.	Analytes	Example
1.	Anesthetic Gases	N <sub>2</sub> O, Halothane
2.	Antigens and Antibodies	Human Ig, Anti-human Ig
3.	Flammable Gases	CH <sub>4</sub>
4.	Ions	H <sup>+</sup> , Li <sup>+</sup> , K <sup>+</sup> , Na <sup>+</sup> , Ca <sup>+</sup> , Phosphates, Heavy Metal Ions
5.	Metabolites	Glucose, Urea
6.	Microorganisms	Viruses, Bacteria, Parasites
7.	Respiratory Gases	O <sub>2</sub> , CO <sub>2</sub>
8.	Toxic Gases	H <sub>2</sub> S, Cl <sub>2</sub> , CO, NH <sub>3</sub>
9.	Toxic Vapors	Benzene, Toluene
10.	Trace Metabolites	Hormones, Steroids, Drugs

In **Table 2** summary of a variety of biosystem-transducer combinations in terms of transducer, measurement mode and potential application. The interaction of antibodies with their corresponding antigens is an attractive reason for attempting to develop antibody-based chemical biosensors, i.e. immunosensors. Theoretically, if an antibody can be raised against a particular analyte, an immunosensor could be developed to recognize it. Despite the high specificity and affinity of antibodies towards complementary ligand molecules, most antibody-antigen interactions do not cause an electronically measurable change. However, the remarkable selectivity of antibodies has fueled much research to overcome this intrinsic problem. The piezoelectric effect in various crystalline substances is a useful property that leads to the detection of analytes.

Table 2- Few Common Biosensor Components

Sl. No.	Transducer System	Measurement Mode	Applications
1.	Conductimeter	Conductance	Enzyme substrates
2.	Enzyme Electrodes	Amperometric	Enzymes, immunological systems
3.	Field-Effect Transistors	Potentiometric	Ions, gases, enzyme substrates immunological analytes
4.	Gas-Sensing Electrodes	Potentiometric	Gases, enzyme, organelle, cell or tissue electrodes
5.	Ion-Selective Electrode	Potentiometric	Ions in biological media, enzyme electrodes
6.	Optoelectronic and Fiber-Optic Devices	Optical	pH; enzymes; immunological analytes
7.	Piezoelectric Crystals	Acoustic (mass)	Volatile gases and vapors, antibodies
8.	Thermistors	Calorimetric	Enzyme, organelle, gases, pollutants, antibiotics, vitamins

Figure 2 shows a schematic diagram of an immunosensor device.[5-14] The piezoelectric immunosensor is thought to be one of the most sensitive analytical instruments developed to date, being capable of detecting antigens in the picogram range. Moreover, this type of device is believed to have the potential to detect antigens in the gas phase as well as in the liquid phase.

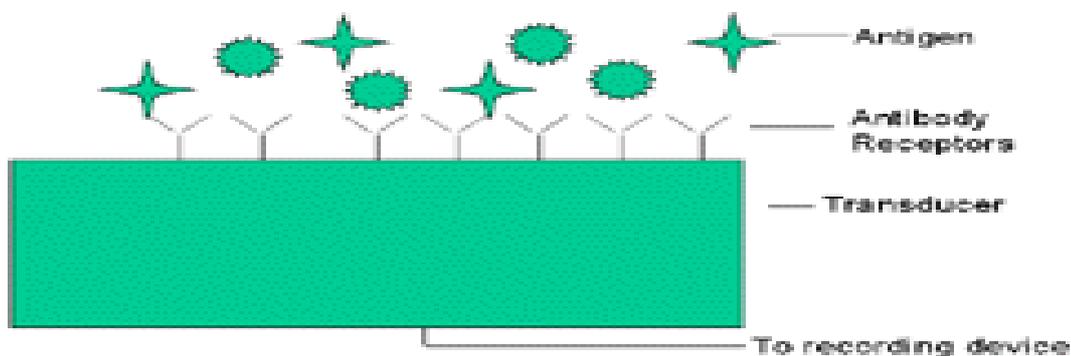


Figure 1. Schematic diagram of an immunosensor device

Almost all current methods of diagnosing tuberculosis (TB) have drawbacks. They tend to be either nonspecific or too time-consuming. In most cases of pulmonary and extra pulmonary TB, diagnosis depends upon culturing the mycobacterial organism, a process requiring 4-8 weeks. Significant attention has been devoted to developing more rapid diagnostic methods for TB, but some of them do not have the high specificity or sensitivity required for proper diagnosis.[15-22]

A piezoelectric sensor that could reliably detect the mycobacterial antigen in biological fluids would be of enormous use. For instance, detection of the antigen in saliva could constitute a noninvasive method of screening high-risk populations. One tested piezoelectric crystal sensor gives results within a couple of hours after exposing the electrode to a liquid containing the antigen. The apparatus would be quite portable, so the immunological tests could be performed virtually anywhere, and the results could be obtained very quickly. The feasibility of using piezoelectric immunosensors to diagnose TB based upon the detection of mycobacterial antigens in liquid depends upon the degree of sensitivity and specificity that can be achieved and upon overcoming any problems caused by potentially interfering substances in biological fluids. The feasibility of gas- or vapor-phase detection of antigen depends upon these same factors, plus any difficulties that may be unique to gas-phase antigen capture by antibodies.

### 3. THEORY AND FORMULATION

The Sauerbrey equation being the most widely accepted for the relationship between the resonant frequency of an oscillating piezoelectric crystal and the mass deposited on the crystal surface. Sauerbrey developed an empirical equation for AT-cut quartz crystals vibrating in the thickness shear mode that describes the relationship between the mass of thin metal films deposited on quartz crystals and the corresponding change in resonant frequency of the crystal:

$$\Delta F = -2.3 \times 10^6 F^2 \frac{\Delta M}{A}$$

Where,  $\Delta F$  = frequency change in oscillating crystal in Hz,  $F$  = frequency of piezoelectric quartz crystal in MHz,  $\Delta M$  = mass of deposited film in g, and  $A$  = area of electrode surface in  $\text{cm}^2$ .

The piezoelectric crystal detector can be a very powerful analytical tool because of the relationship shown for the change in frequency to the analyte concentration with high sensitivity. Conversely, the above explanation shows that the crystal detector indiscriminately changes frequency due to the deposition of mass of any material on its surface. Thus, it is the task of the researcher to choose a coating that will undergo a highly selective chemical or physical binding with the substance to be detected. Only then can a highly selective sensor be constructed that will be sensitive to the subject to be detected.

### 4. EXPERIMENTAL PROCEDURES

The most frequently used detector crystal is alpha quartz. These crystals are most suitable for piezoelectric application because they are insoluble in water and resistant to high temperatures. Alpha quartz crystals can be resistant to temperatures up to 579°C with no loss of piezoelectric properties. The resonant frequency of quartz

crystal depends on the physical dimensions of the quartz plate and the thickness of the electrode deposited. AT and BT-cut crystals are most useful as piezoelectric detectors. These cuts refer to the orientation of the plate with respect to the crystal structure. The AT-cut crystal is the most stable, with a temperature coefficient of 1 ppm per degree centigrade over a temperature range of 10°C to 50°C. The crystals usually take the form of discs, squares, and rectangles.

All crystals in this investigation were general-purpose 10 MHz AT-cut quartz crystals with an electrode coating deposited on each side using sputtering method. The crystal was mounted on a holder with stainless steel with leads. Silver composite was used to connect the electrode to wire. The crystals were 14 mm in diameter, and the electrodes on both sides of the crystal were 8 mm in diameter. The crystals were mounted on size HC6/u holders. Figure 2 shows the schematic diagram of the fabricated crystal attached to the base.

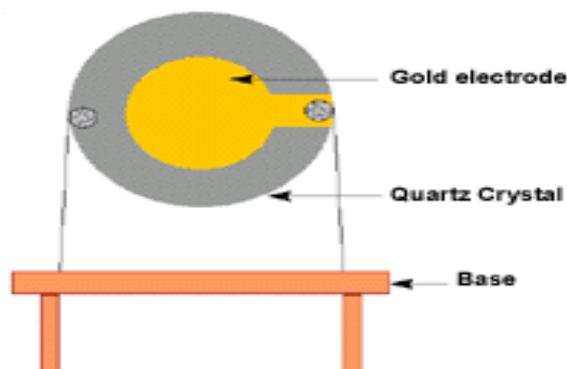


Figure 2. Quartz crystal and holder

Figure 4 is a block diagram of the apparatus used for the biosensor experiment. The piezoelectric quartz crystal was driven by a low-frequency transistor oscillator, powered by a 1-30 V D.C. regulator power supply and set at 9 V d.c. The frequency of the vibrating crystal was monitored by a Protek multifunction frequency counter. The crystal mounted on its holder was connected to the oscillator circuit and the frequency counter was connected to the oscillator device. After each step in the coating process—first with the various metal depositions and then with the bio molecular analytes—the frequency reading was recorded.

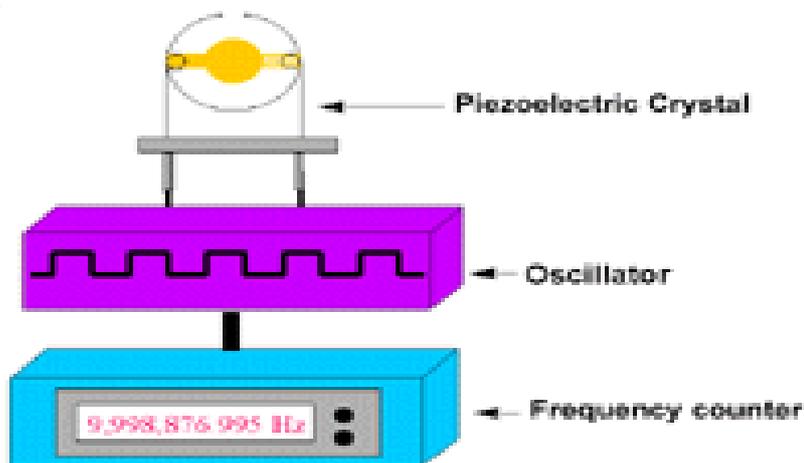


Figure 3. Experimental apparatus for a piezoelectric sensor

## RESULTS AND DISCUSSION

The use of magnetic materials underneath the gold coating helped make the antigen detectable. Although platinum is a more noble material compared to the gold, the adherence of the platinum films was very poor on quartz substrates and the platinum reacted with different buffer solutions, for examples hydrochloric acid and sodium hydroxide during the specimen preparation for antigen-antibody binding. Thus, gold electrodes are preferred due to superior adhesion and non-reactive properties. The optimum thickness of the gold electrode layer was estimated at 1,000 Angstroms. Although the binding between antigen and antibody did show a change in frequency, the results were not always reproducible. The antibody binding to the protein layer was critical to achieve desirable results. [23-25]

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