

*Short Review*

## **The Piezoelectric Biosensors: Principles and Applications, a Review**

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Piezoelectric materials have broad use in many technological applications and they are frequently embedded in electronic devices. In principle, the piezoelectric materials work as oscillators on piezoelectric effect principle and interaction with their surface is easily detectable hence they are well suitable for construction of biosensors recognizing affinity interactions. Assays based on reaction between antigen and antibody, two polynucleotide strains, aptamer and protein are well suited for such studies. The current review is focused on explaining of the piezoelectric biosensors function, data about available materials and examples of analytical applications. Survey of actual literature is provided here.

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**Keywords:** acoustic sensor; piezoelectric; quartz crystal microbalance; QCM; anisotropy; biosensor; oscillation; label free; immunosensor

### **1. INTRODUCTION**

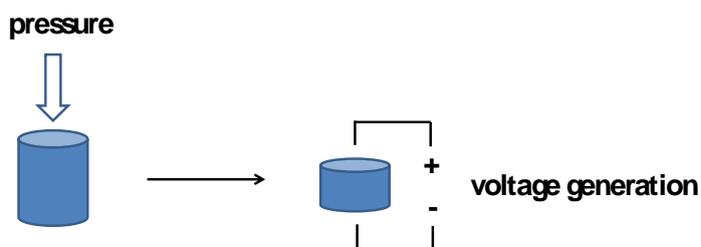
Biosensors are miniaturized devices composed from the sensor part known also as physico-chemical transducer and a part of biological origin like antibody, enzyme, nucleic acid sequence, organelle, viable cell or slice of a tissue [1,2]. We can entitle as the first biosensor the device constructed by Clark and coworkers in early 1960s [3,4]. The inventors constructed a voltammetric analyser having enzyme glucose oxidase tightly connected with the surface of working electrode. Because the device had the both BIOlogical origin part and electrochemical SENSOR, acronym biosensor has appeared afterwards.

Many types of sensor platforms can be chosen when a biosensor construction is intended. We can learn about voltammetric, potentiometric, optic, non-linear optic and piezoelectric platforms

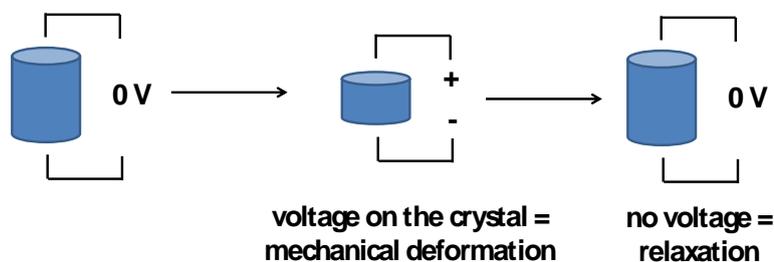
suitable biosensors when the current literature searched [5-9]. In this paper, piezoelectric biosensors are extensively reviewed as an important tool suitable for direct assay of analytes via label free affinity interactions. Overall simplicity and low price of piezoelectric sensors are favorable for practical use. Principles of the biosensors function, pros and cons of the assays and survey of examples are given here.

## 2. PRINCIPLE OF PIEZOELECTRIC EFFECT

Piezoelectric effect is not a completely novel idea since it has been known since the 19<sup>th</sup> century with broad technological applications since the beginning of the 20<sup>th</sup> century. The discovery of piezoelectric effect is connected with the names of famous physicists Jacques Curie and Pierre Curie who recognized that anisotropic crystals i.e. crystals without center of symmetry can generate electric dipole when mechanically squeezed. The electric dipole is also called piezoelectricity. The described effect can work in oppose way when an anisotropic crystal become deformed due to voltage imposed on it [10,11]. The aforementioned phenomenon is depicted as figure 1. The mechanical deformation is, however, a simple situation and oscillation is rather chosen in the common applications like here described analytical devices [12]. In the case of oscillation, an alternating voltage is imposed on the crystal and mechanical oscillation then occurs.



**Figure 1.** Piezoelectric effect when voltage is generated because of mechanical deformation



**Figure 2.** Piezoelectric effect when mechanical deformation is initiated by an applied voltage

The oscillations can have many appearances depending upon material and other conditions like electrical contacts, shape of the crystals etc. The oscillations occur in adiabatic waves which are typically spread over the mass like the acoustic one. In the oscillating crystals, the both surface

acoustic waves spreading on the material [13] and bulk acoustic waves occurring in deep matter [14] can take place.

In standard analytical applications, frequencies of oscillations are measured and interaction with either crystal alone or electrode leading electricity impulse on the crystal surface can serve for the determination of analyte. The bound mass on the crystal surface causes slowing of oscillation. For the common quartz crystals, the frequency  $\Delta f$  shift is directly proportional to mass  $\Delta m$  bound on the crystal which was described by Sauerbrey as follow [15,16]:

$$\Delta f = \frac{-2f_0^2 \Delta m}{A\sqrt{\rho_q \mu_q}} = -2.3 \times 10^6 f_0^2 \frac{\Delta m}{A}$$

In the equation,  $f_0$  means the fundamental mode of the crystal oscillation in hertz and  $A$  is the piezoelectrically active area in centimeters. The symbol  $\rho_q$  means density ( $2.648 \text{ g/cm}^3$ ) and  $\mu_q$  means shear modulus ( $2.947 \times 10^{11} \text{ g/cm} \times \text{s}^2$ ) of quartz. Though the Sauerbrey equation exactly describes interaction with a mass, there is also a drawback limiting its use for an analytical application because not only physical or chemical interactions but also other properties like viscosity of ambient solution has impact on the frequency shift. The Sauerbrey equation is a reliable description of a rigid, thin-film firmly attached on the electrode surface but when ambient environment is not unaltered, equation described by Kanazawa and coworkers for quartz crystal should be taken into consideration [17,18]. In a brief description, the equation states that frequency shift is proportional to increase of ambient viscosity  $\eta$ :

$$\Delta f = f_0^{3/2} \sqrt{\frac{\Delta(\rho_l \eta_l)}{\pi \rho_q \eta_q}}$$

The symbols meaning is the same like for the Sauerbrey equation, the symbols with index “l” related to the ambient liquid and “q” to quartz crystal. The fact that viscosity has impact on frequency shift has to be taken into consideration during any assay. For instance, a sample with analyte being solved in an organic solvent will have other fundamental frequency in given condition than basic water based buffer even when no interaction of analyte and crystal surface take place. Samples with different viscosities then provide different oscillation which can be easily misinterpreted [19]. Contemporary assay with a modified crystal and blank crystal is one of the countermeasures. The determination of other parameters like can be also helpful [20].

### 3. MATERIALS FOR PIEZOELECTRIC ASSAYS

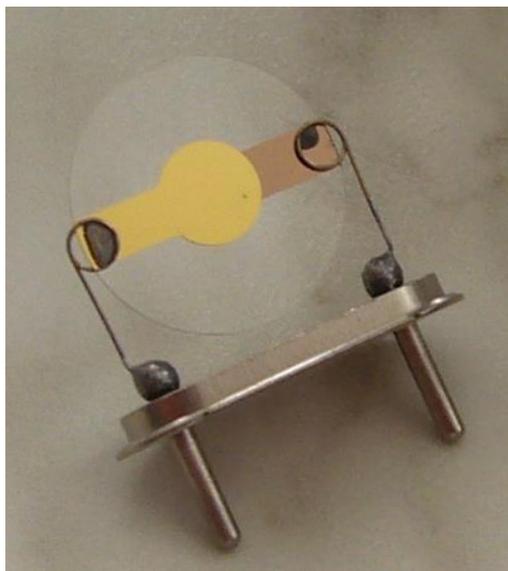
There is high number of anisotropic materials exerting piezoelectric effect. Inorganic, organic and even some biomolecules like nucleic acid can provide piezoelectricity. On the other hand,

construction of biosensors is linked with not so high number of materials and quartz crystal is probably the most common because of reliability and good availability. Further changes can be expected in the future because new material synthesis and nanomaterials construction.

Aluminium phosphate also known as berlinite, aluminium nitride, zinc oxide, crystalized topaz, crystalized tourmaline, barium titanate, gallium orthophosphate, lead titanate and quartz  $\text{SiO}_2$  are typical examples of inorganic anisotropic materials [21-27]. Organic materials exerting piezoelectric effects can be also mentioned as applicable source for biosensors construction. Sodium potassium tartrate tetrahydrate known as Rochelle salt can be exemplified as the organic material [28,29]. Organic polymers like polyvinylidene fluoride, and optically active one like polyamides and polylactic acids are synthetic materials manifesting piezoelectric effect [30,31]. Hybrid films containing polyvinylidene fluoride as crucial part have broad application potential [32-34].

Piezoelectric effect is known for biological molecules as well which bring new opportunities in biosensors constructions. Protein ion channels with anisotropic properties are such promising group of biological molecules deserving further investigation [35,36]. A murine protein Piezo1 channel composed from 2,547 amino acids and weight 900 kDa is also a macromolecule with piezoelectric properties [37,38]. Piezo channels involved in mechanosensory of nociception are described also in *Drosophila* [39]. Collagen is another protein based biomolecule which piezoelectric properties are known [40,41]. Piezoelectric properties can be found in sugars. Cellulose is one of the most available sugars for which piezoelectric properties were revealed [42,43].

As a platform for biosensors construction, quartz crystal microbalances (QCM) are very popular tool because these type of sensor is mass produced for electronic industry hence price is quite low and the sensor can be easily purchased from any of manufacturers. Currently, they are used as attenuators in electronic devices and they have typically fundamental mode frequency 1 – 20 MHz. The common manner of a quartz manufacture processing is done by an AT cut giving opportunity to operate on frequencies between 0.5 and 300 MHz but other cuts are available (BT, CT, DT, ET, FT, GT, IT, SC, XY ...) [44-47]. The sensitivity of a QCM is for instance  $56.6 \text{ Hz} \times \mu\text{g}^{-1} \times \text{cm}^2$  for a QCM with the fundamental frequency 5 MHz [48]. The calculated sensitivity for a 50 MHz sensor is  $5.657 \times 10^9 \text{ Hz} \times \text{g}^{-1} \times \text{cm}^2$  respective  $5,657 \text{ Hz} \times \mu\text{g}^{-1} \times \text{cm}^2$  when expressed in the same units like the previous reference [49]. When compared the sensitivities from the two papers it is obvious that increase of the fundamental frequency 10 times will cause 100 times higher sensitivity to a mass bound of the sensor surface. Though the higher frequencies provides good opportunities for a sensitive assay, the QCM with the high frequencies have also drawbacks like fragility and technologically demanding equipment for manufacturing. In order to achieve oscillations, voltage should be laid on the oppose electrodes on the QCM. The common materials technologically used for the electrodes are gold and silver but other noble metals can be chosen for the construction purposes. Picture of a common QCM sensor is presented in figure 3.



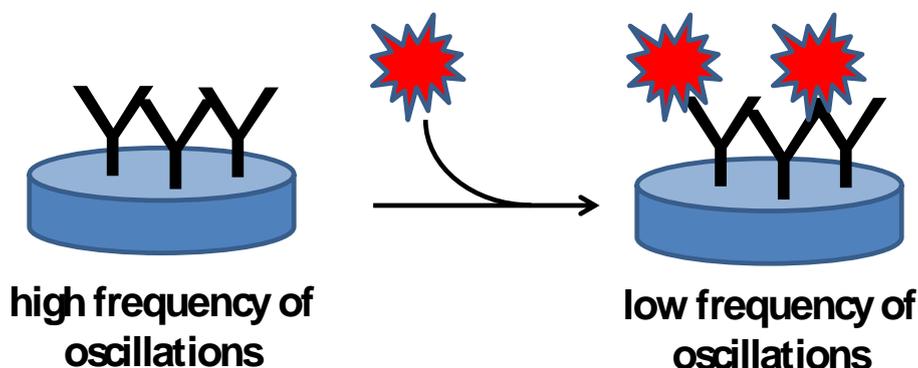
**Figure 3.** A QCM sensor with fundamental mode frequency 10 MHz and surface covered with gold electrodes on the both opposite sides.

#### 4. BIOSENSORS CONSTRUCTION

Piezoelectric biosensors can work in several modes from which direct, label free, interaction with analyte provides the maximal use of advantages offered by the piezoelectric platform. In this way, antibodies and antigens appear as promising biomolecules well compatible with a piezoelectric sensor. We can explain the idea on two examples where interaction of antibody – antigen is a necessary part of the assay. In the first example, we can discuss biosensor with immobilized antibody and suitable for the determination of whole bacterial cells [50]. In this experiment, polyclonal antibody against *Francisella tularensis* was prepared by immunization of white BALB/c mice and the antibody was immobilized on surface of a 10 MHz QCM. When *F. tularensis* was presented in examined sample, then arose interaction of antigen – antibody and the bacterium was attached to the surface followed with change of frequency up to 40 Hz. The complex bacterium – antibody can be split by extreme pH resulting in re-usability of the biosensor. Other bacteria like *Escherichia coli* and *Bacillus subtilis* underwent no interaction with the biosensor surface. Though the biosensor worked well, the change of frequency was quite low. Principle of a simple biosensor with immobilized antibody is depicted as figure 4.

In another application, 10 MHz QCMs were used for a fast and label-free determination of tularemia, an infectious disease caused by *F. tularensis* [51]. The experiment was very close to the previous because interaction antigen antibody takes place here. However, the used QCM had immobilized antigen on its surface and antibodies against *F. tularensis* were assayed as a diagnostic marker of tularemia disease. When analyzed serum samples from infected hares, maximal change of oscillations equal to approximately 200 Hz was achieved. The biosensor well correlated with the standard enzyme-linked immunosorbent assay and was presented as simple tool for reagent-less

diagnosis of the disease. When compared the two examples it can be seen that the significantly smaller antibodies provided better signal when compared to the biosensor for bacteria. It is probably caused by the fact that the bacteria do not behave like a point particle. Decay of oscillations is caused by antigen determinant and close region in cell wall, the farther parts behave like viscous medium rather than point particle.



**Figure 4.** Simplified scheme of an immunosensor having surface covered with antibodies (dark “Y”) and interacting with an antigen (red asterisk). Blue barrel represent an anisotropic crystal.

The QCM biosensors are not suitable for antigen-antibody interactions only. Other types of macromolecules can be chosen due to their properties. A QCM biosensor with immobilized DNA served for recognizing of genetically modified organisms via hybridization resulting in mass increase on the biosensor surface [52]. His tagged proteins can directly interact with the sensor surface hence these macromolecules can be assayed by the interaction. Li and coworkers used the His-tag interaction with metals and assayed the proteins on a QCM biosensor [53]. An immunosensor having 10 MHz AT cut based QCM was covered with antibodies against okadaic acid was performed for the acid assay in food samples [54]. The direct interaction was found to be reliable and the interaction was followed with decrease of oscillations up to 900 Hz.

Apart of commercial sensors, the piezoelectric biosensors can be made on in-lab synthesized materials. Thin and highly flexible membranes with piezoelectric properties synthesized Spanu and coworkers from polyvinylene fluoride [55]. Zhao and coworkers synthesized nanowires from  $\text{SiO}_2$  with surface covered with ZnO and successfully performed them for the determination of immunoglobulins G [56]. A prototype sensor based on  $(\text{K},\text{Na})\text{NbO}_3$  microstructured fibers was fabricated like a platform promising for analytical and diagnostic purposes [57]. Other opportunity to perform the piezoelectric sensors is to cover their surface by molecularly imprinted polymers resulting in possibility to determine interactions close to the antibody – antigen but with replacement with the antibody by the imprinted polymer [58]. This improvement can not only reduce price of the biosensor but also provide better reproducibility of manufacturing process.

This review is focused on piezoelectric biosensors. There is a group of biosensors close to the piezoelectric biosensors: piezoresistive one. In their principle, the biosensors work on principle of

change in electrical resistivity due to mechanical stress. The piezoresistive materials are very intriguing and they give opportunity to construct cantilever shaped sensors. In an example, microcantilever based biosensor was made by Agarwal and coworkers as a substrate for the determination of the both biological and chemical analytes [59]. Microelectromechanical sensor was made by Dhakane and Patil as a tool for the revealing of tuberculosis [60]. The piezoresistive biosensors are, however, out of this review scope which is primary devoted to the piezoelectric one.

## 5. THE EXAMPLES OF ASSAY

In this chapter, applications of piezoelectric biosensors are depicted. We can start with the application made by Babacan and coworkers for the determination of *Salmonella typhimurium* [61]. The authors immobilized antibodies against *S. typhimurium* via protein A on a QCM with surface pre-coated with polyethyleneimine. An interaction with *S. typhimurium*  $1.5 \times 10^9$  CFU/ml caused change of oscillations equal to approximately 50 Hz. QCM based biosensor constructed on a base of a AT cut with basic frequency 9 MHz and with gold electrodes was constructed for the determination of hepatitis B virus [62]. The gold electrodes were modified with polyethyleneimine and hepatitis DNA was immobilized by crosslinking with glutaraldehyde. Interaction of the DNA on sensor with hepatitis in sample allowed detection of hepatitis DNA from 0.02  $\mu\text{g/ml}$ . Piezoelectric biosensors are suitable also for rapid detection of HIV in biological fluids [63-65]. Quartz cut as a part of thickness shear mode resonator with basic frequency 50 MHz served for the construction of an immunosensor suitable for the determination of surface glycoprotein gp120 from HIV [66]. The immunosensor contained an antibody against gp120 immobilized on gold electrode of the quartz cut. As low as  $6.5 \times 10^4$  viruses/ml for a biological fluid sample sized 5  $\mu\text{l}$  can be analyzed by the biosensor. In another application, wafers produced by lithography were made from silicon dioxide and consequently antibodies against gp24 from HIV-1 and gp39 from HIV-2 were deposited on the wafer surface [67]. The biosensor detected 3,000 viral particles in a serum sample and the assay correlated with standard polymerase chain reaction. Piezoelectric biosensors for HIV can also work in oppose mode i.e. with immobilized antigen and suitable for the diagnosis of disease by detection of antibodies specific to HIV [68]. Other causative agents of infectious diseases can be determined by piezoelectric biosensors. *Mycobacterium tuberculosis* was assayed by a 12 MHz AT cut of a quartz crystal with gold electrodes [69]. The gold electrode was modified to form self-assembled monolayer containing oligonucleotide specific to the *M. tuberculosis*. The sensitive surface then interacts with real samples with proved accuracy when compared to polymerase chain reaction.

A QCM based biosensor was constructed for the determination organophosphorus and carbamate pesticides [70]. The sensor had immobilized enzyme acetylcholinesterase which converted 3-indolyl-acetate to insoluble indigo pigment providing alteration in the oscillations. The biosensor was used for the assay of pesticides which inhibit the enzyme acetylcholinesterase. When the enzyme became inhibited, the precipitate was not formed. As low as  $5 \times 10^{-8}$  mol/l of paraoxon and  $1 \times 10^{-7}$  mol/l of carbaryl were assayed. Piezoelectric biosensors are also a promising tool for the revealing of protein markers. Metalloproteinase-9 is a zinc dependent hydrolase releasing because of cellular matrix

degradation and released when e.g. cardiovascular dysfunctions appear. Scarano and coworkers constructed biosensor having aptamer as a biorecognition element [71]. The biosensor was based on an AT cut of 9.5 MHz QCM with gold electrodes covered with the aptamer. When the biosensor performed, limit of detection equal to 1.2 pmol/l of metalloproteinase-9 was reckoned from calibration. Survey of the aforementioned assays by biosensors is provided in table 1.

**Table 1.** Assays by piezoelectric biosensors

Piezoelectric platform	Analyte	Principle of the assay	Reported limit of detection	References
9 MHz QCM with gold electrodes	hepatitis B virus	hybridization of hepatitis DNA with DNA immobilized on the QCM	0.02 µg/ml	[62]
50 MHz quartz with gold electrodes	antigen gp120 from HIV	interactions of immobilized antibody with the gp120 located on surface of HIV	$6.5 \times 10^4$ viruses/ml	[66]
silicon wafer manufactured by lithography	gp24 from HIV-1 and gp39 from HIV-2	interaction of an antibody immobilized on sensor surface with antigen on surface of HIV	3000 viruses	[67]
An QCM	organophosphorus and carbamate pesticides	immobilized acetylcholinesterase provided precipitate which was piezoelectrically visible, in presence of the pesticides, the enzyme was inhibited and no precipitate formed	$5 \times 10^{-8}$ mol/l of paraoxon, $1 \times 10^{-7}$ mol/l of carbaryl	[70]
9.5 MHz QCM – AT cut	metalloproteinase-9	interaction between aptamer on the biosensor and analyte	1.2 pmol/l	[71]

## 6. CONCLUSION

Piezoelectric biosensors are available devices suitable for the determination of analytes by affine interactions without application of any further reagents. When compared to the methods like surface plasmon resonance having the same feature, piezoelectric biosensors can be manufactured and performed with quite low costs and simple assay devices. Especially diagnosis based on the

determination of macromolecules can attain popularity for piezoelectric biosensors in the modern bioanalysis. Further research on piezoelectric materials can enhance potential for routine applications.

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