Biosensors based on piezolectric transducers

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In this paper, basic principles of piezoelectric transducers are presented. Various systems are devised and a concrete example is exposed for direct biodetection with a 27 MHz QCM. Endly, recent and typical applications, using classical QCM for biosensing, are summarized

Introduction

The development of immunosensors based on piezoelectric transducers is widely investigated due to their attractive applications in mass sensitive detection [1, 2]. The most classical transducer is based on the quartz crystal microbalance (QCM), adapted to the liquid medium, which gives a direct response signal which characterizes the binding event between a sensitive layer, grafted onto the surface transducer, and the analyte to be detected. This kind of transducers offers many applications and an increasing number of publications illustrates this phenomena [3]. However, for small biomolecule detection under minute concentration it is quite difficult to obtain an observable and direct signal. This is mainly due to the lack of mass sensitivity of the commonly used QCM as they are generally built with 5 to 10 MHz quartz crystals. For solving this problem, attemps were made by increasing the working frequencies of the devices as, in general, the mass sensitivity increseases at the same time. Several possibilities were examined : either by using classical QCM with a smaller crystal thickness which increased the resonant frequency or by developping interdigitated piezoelectric devices at higher working frequencies. Various mass sensitivities will be calculated in order to compare the mass sensitivities of the different piezoelectric transducers. Moreover, we insists on the part played by other physico-chemical effects than the mass and which affect the piezoelectric response.

As examples, the feasibility of these devices was demonstrated with peroxidase and staphylococcal enterotoxin B as

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detected antigens where the selective layer was obtained by adsorption of the corresponding antibodies onto the 27 MHz QCM transducer. We pointed out the problem for testing the specificity of the biosensing for these examples. Endly, other recent applications of the QCM for biodetection were given.

Piezoelectric transducers

Generalities

The piezoelectric transducers allowed a binding event to be converted into a measurable signal, for example resonance frequency changes. The principle was based on the piezoelectric properties of some material such as quartz crystals. Indeed, if an electrical field was applied through quartz, the inner dipoles were reorientated and a crystalline mechanical strain was observed. In 1920, Cady used the reverse piezoelectric effect for realizing high stable oscillators incorporating quartz resonators : under an alternate electrical field, mechanical vibrations of the crystal were observed and ultrasonic waves were generated, the quartz crystal vibrating near its resonant frequency. When it was included in an appropriate electronic circuit, the measured oscillation frequency was closed to the resonant frequency and the generated wave amplitude reached a maximum. Thus, a modification of a physical characteristic of the resonator, for example the global mass or the thickness, led to a resonant frequency variation. For biosensors, mass changes, occuring from the interaction between the modified transducer surface and a detected species, can be measured by this way. Several piezoelectric devices were developped based on this principle and three kinds of transducers were now presented.

Piezoelectric devices

TSM

TSM devices were based on the acoustic wave propagation under a thickness shear mode (TSM) inside a quartz plate (Fig. 1). These transducers were called Quartz Crystal Microbalance (QCM) and were developped twenty years ago for liquid applications [4, 5]. The active element of a QCM was formed by a thin round plate of quartz with two gold electrodes deposited on the two opposite sides (Fig. 2). These electrodes allowed the alternate electrical field to be applied. The quartz crystal oscillated near its own resonant frequency and was directly dependent on the quartz thickness. When a mass was added or removed, it was considered as an increase or a decrease of its thickness. Thus, a mass change onto the exciting electrodes led to a frequency shift easily measured through the oscillator signal. In general, the classical working frequencies for biosensors are ranging between 5 MHz to 10 MHz. These devices appeared well adapted for realizing biosensors due to their low cost and their simplified technology.

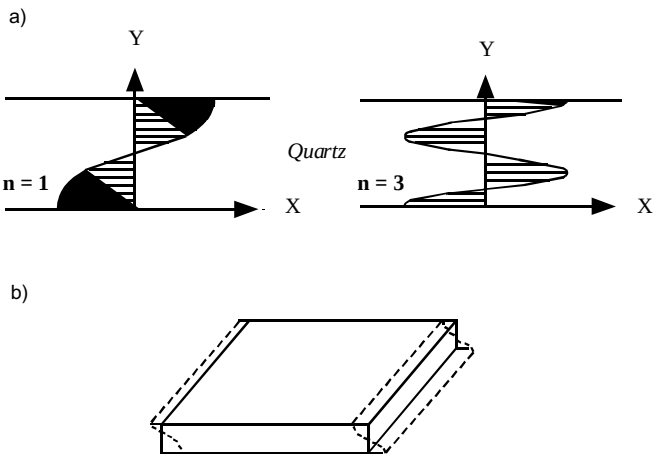


Figure 1. a) schematic representation for a thickness shear wave device in the fundamental mode (n=1) and in the third overtone (n=3) b), vibration for a quartz plate in thickness shear mode (TSM).

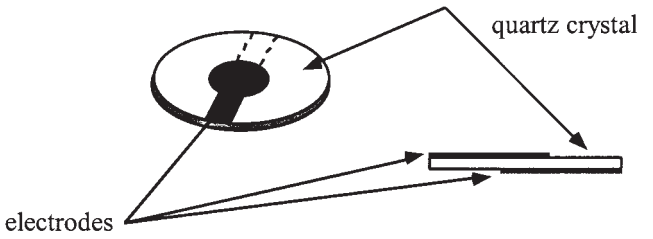


Figure 2. QCM quartz crystal with two gold electrodes.

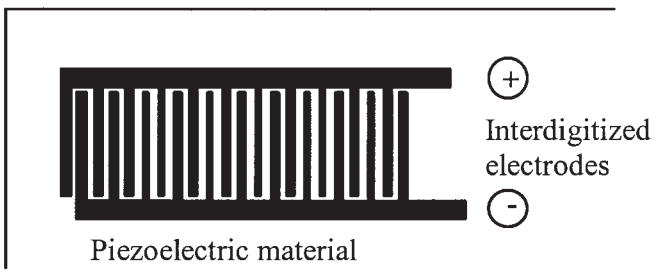


Figure 3. Example of interdigitized electrodes deposited onto a piezoelectric material.

SAW

SAW devices, surface acoustic waves, are based on the interdigitized electrode technology and allowed the generation of surface waves. Figure 3 shows one example of configuration. These transducers are based on the study of the perturbation of the propagation rate of the surface wave (Rayleigh wave) between two pairs of interdigitized electrodes. This rate is dependent on the piezoelectric material

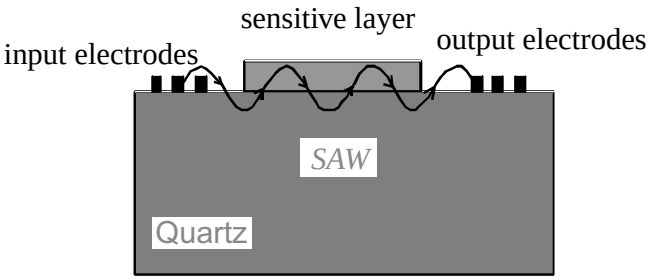


Figure 4. Schematic representation of a surface acoustic wave (SAW) device.

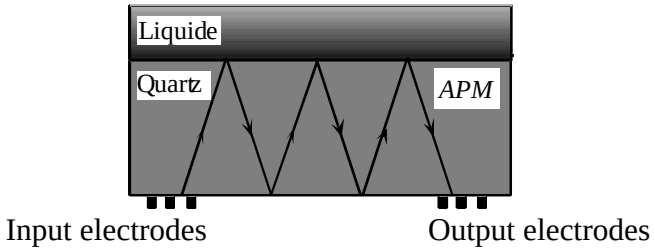


Figure 5. Cross section of a typical APM transducer showing the substrate, the interdigitized electrodes and the fluid sample.

and on the crystal cut. The frequency is calculated from the width of the gap between each interdigitized electrodes. Figure 4 presents a typical SAW device called in this configuration, "delay line". When the sensitive layer becomes more heavier, the propagation rate decreased proportionally to the added mass. A large range of frequencies are used (30 to 200 MHz) which allowed very high mass sensitivities [6]. Unfortunately, these devices do not work properly in contact with a liquid due to strong radiation loss into the liquid. An other kind of surface acoustic transducer was developped for liquid applications. It is called SH-SAW (Shear Horizontally polarized Surface Acoustic Wave) and works with lithium tantalate [7, 8] as a piezoelectric material.

APM

This device was developped recently and was constitued with a quartz plate. It allowed the sound wave to be propagated inside the material. As before, interdigitized electrodes are used, a cross section of a typical APM delay line was presented in figure 5. This apparatus could be employed under gas phase or in contact with a liquid. The waves were propagated through the material and reflected between the two opposite sides of the plate. Decrease the plate thickness led to increase the resonance frequency. The working frequency range was between 25 to 200 MHz [9, 10].

Basic principle of the QCM

Sauerbrey equation

In a first step, investigation of the mass effect was made by Sauerbrey [11] who derived the relationship between the change in resonance frequency and the added mass. This equation was valid only for thin, uniform and purely elastic added layers.

The demonstration was based on the equivalence between the quartz crystal thickness and the resonance frequency by the following relationship:

$$e = \frac{n\lambda_n}{2} \quad (1)$$

where e is the quartz thickness, n, the overtone number and λ_n the wave length.

Moreover, the quartz thickness is related to its mass by:

$$e = \frac{m}{A\rho} \quad (2)$$

where m is the mass of the crystal, A is the active area and ρ the quartz density (2.648 $g.cm^{-3}$).

By combining equations (1) and (2) and by assuming an increase of the quartz mass small compared with the quartz mass, the change of frequency, Δf, is:

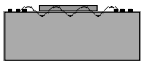
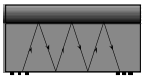
$$\Delta f_n = -\frac{1}{N\rho} \times \frac{f_n^2}{n} \times \frac{\Delta m}{A} \quad (3)$$

where f_n is the resonance frequency of the unloaded resonator, N the acoutic wave speed divided by two

Table I. Theoretical sensitivity for various QCM according to the resonance frequency.

Resonance frequency/MHz	*Crystal thickness in µm*	*Theoretical sensitivity coefficient calculated from the sauerbrey equation in Hz.g^{-1}.cm^2*	*Gain compared with a classical 6 MHz*
6 fundamental mode	278	$8{,}14 \times 10^7$	-
9 fundamental mode	185	$18{,}31 \times 10^7$	x2,25
27 (overtone 3)	185	$54{,}95 \times 10^7$	x6,75
27 fundamental mode	62	$164{,}85 \times 10^7$	x20,25

Table II. Mass sensitivities for various piezoelectric devices, comparison with the different calculated coefficients.

Device	*Sensitivity coefficent* k *in Hz. g⁻¹. cm²*	Δm *equivalent to 1 Hz for* Δf *for a defined resonance frequency* (f)
		f = 6 MHz → 12,3 ng.cm^{-2}
	$k = -2{,}26.10^{-6} \times \frac{f_n^2}{n}$	*f* = 27 MHz (9 MHz overtone 3) → 1,8 ng.cm^{-2}
SAW 	$k = -2{,}26.10^{-6} \times f^2$	*f* = 200 MHz → 0,01 ng.cm^{-2}
APM 	*k* = –20*f* (pour n=1)	*f* = 104 MHz → 0,5 ng.cm^{-2}

(3340 m.s^{-1}), *A* the active area (delimited by the exciting electrodes) and *Δm* the mass change. Thus for the quartz, numeric application led to :

$$\Delta f_n = -2{,}26.10^{-6} \times \frac{f_n^2}{n} \times \frac{\Delta m}{A} \qquad (4)$$

Table I gives various frequency/mass senstivities depending on the resonance frequency of the device.

A comparison of the mass sensitivities of the various devices described previously are presented in table II. The SAW device appears as the most sensitive mass transducer, its limit of detection can reach 10 pg.cm^{-2} but SAW can be applied only in gas phase. Between APM and QCM, the difference is not so large especially if the resonance frequency of the classical QCM system is increased.

Sauerbrey relationship limitations

These limitations were in general due to a non ideal behaviour of the added film.

A first problem became for a non uniform added layer. Several works [12, 13, 14] have shown a large effect over the QCM response for localized deposits because the mass sensitivity is radius dependant : it is better in the center of the QCM than on the outer edge of the electrode. An other point concerned the effect of roughness over the QCM response as biosensors works in liquid phase. The surface morphology changes affected the QCM response by changing the coupling between the liquid and the active surface [15]. Theoretical approaches were developped [16, 17]; approximate modeling were generally given due to difficulties to characterize the sound wave near the rough surface/liquid interface.

Martin [18] shew also that changes of the liquid properties (viscosity, density of the solution) affected the QCM response. In the case of small added mass, in a Newtonien liquid, the contribution due to the added mass and to the liquid are additive. By considering an added mass rigidly coupled to the quartz surface, a continuous waves at the film/solution interface and a semi infine liquid thickness the change of frequency is equal to:

$$\Delta_f \cong -\frac{2f_s^2}{n\sqrt{c_{66}\,\rho_q}}\left[\rho_f h_f + \left(\frac{\rho_L \eta_L}{4\pi f_s}\right)^{1/2}\right] \qquad (5)$$

where f_s is the series resonance frequency, n the overtone number, c_{66} the stiffness modulus, ρ_L the liquid density, ρ_q the quartz density, ρ_f the film density, η_L the liquid viscosity and h_f the film thickness.

By considering only the liquid effect, models were developped based on physical propagation of the sound wave inside the liquid [19, 20]. Kanazawa proposed an original approach based on the coupling between the stationnary shear waves and the damping wave which propagates in the liquid medium. The frequency change is equal to :

$$\Delta f = -f_q^{3/2}\left(\frac{\rho_L \eta_L}{\pi \mu_q \rho_q}\right)^{1/2} \qquad (6)$$

where ρ_q and ρ_L are, respectively, the quartz and the solution densities, η_L the dynamic viscosity, μ_q the quartz shear stiffness and f_q the resonance frequency.

The influence of the viscoelastic properties of thin films was also studied. Experiments were carried out for various materials and the contribution of the shear modulus changes of these films was investigated [21, 22, 23]. A dependence of the electrical response of film coated acoustic wave sensors on the viscoelastic properties of the added layer were observed. A new concept of measurement was necessary to extract complementatry informations : impedance analysis of the loaded resonator. Various models were proposed : on the one hand, equivalent circuit approaches [24, 25, 26, 27] obtained by network analysis, where unfortunately real physical processes were masked and on the other hand, models based on the fundamental equations of transverse motion and electricity [28, 29, 30]. In the latter case, a most accurate representation was obtained and the different calculations were simplified by using transmission line theory [21, 22].

Piezoelectric transducers for biosensing

Ultrasensitive QCM for immunosensing

Immunosensors based on piezoelectric transducers were already widely studied due to their attractive potentialities

[31, 32, 33, 34]. The quartz crystal microbalance (QCM) adapted to liquid media may give a direct response signal characterizing the binding event between a sensitive layer, for example grafted onto the surface transducer, and the analyte to be detected. Direct measurements are attractive compared with classical colorimetric tests, such as ELISA, where several steps are necessary to obtain an optimal signal, which are, of course, time consuming. However, to detect small biomolecules, such as antigens, it is quite difficult to obtain an observable and direct signal; in general intermediate steps of amplification and dip and dry techniques are necessary [35, 36]. This is mainly due to the lack of sensitivity of classical QCM which are generally built with 5 to 10 MHz quartz resonators [37, 38]. Moreover, techniques for immobilizing antibodies are very often difficult to carry out and sometimes cannot be easily reproduced [35, 39]; in general, this is due to involved chemical reactions included in the experimental procedure.

An application, where the mass sensitivity was increased by increasing the resonant frequency of the QCM to 27 MHz was proposed [40, 41]. A new cell, incorporating 27 MHz quartz resonator was devised to continuously measure immunoreactions in liquids. The feasibility was demonstrated by detecting two different biospecies [42, 43].

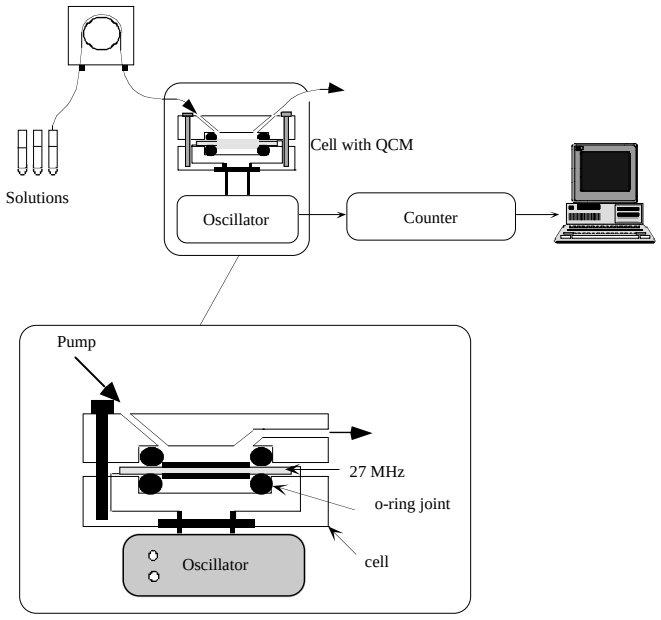


Figure 6. Experimental set-up with the complete system (pump, cell and QCM) for immunosensing at 27 MHz with a QCM.

Piezoelectric transducer and test cell

AT-cut planar quartz crystals (14 mm in diameter) with a 9 MHz nominal resonance frequency (CQE, France) was used. Two identical gold electrodes, 2000 Å thick and 5 mm in diameter, were deposited, by evaporation techniques, on both sides of crystals with a chromium underlayer. The resonators were carefully cleaned in two solvents, acetone and ethanol (Merck, analytical grade), one after the other, for two minutes in an ultrasonic bath. Then, they were dried under pure nitrogen and connected with a silver conducting paste, through wires, to BNC connectors. An home made oscillator was designed to drive the crystal at 27 MHz (9 MHz crystal used on the third overtone). To improve the stability, all the electronic oscillator components were temperature controlled through a heating current monitor (Watlow, USA) with stability better than 0.1 K.

An experimental cell was also developped : the crystal was mounted between two O-ring seals inserted in a Plexiglas cell (figure 6). Only one face of the quartz was in contact with the solution. The cell volume was about 50 µl and the apparatus includes a peristaltic micropump (P1, Pharmacia) to assure a constant flow (60 µl min^{-1}) of the solution on the working quartz crystal. The experimental set up was built by coupling an home made QCM and a frequency counter (Philips PM 6685) in order to follow the microbalance frequency during the biomolecule binding with the sensitive layer.

Transducer preparation

Mouse monoclonal antibodies against peroxidase (Sigma) were immobilized by direct adsorption onto the surface transducer. The methodology was the following : 100 µl of an antibody solution (100 µg ml^{-1}) was deposited onto gold and incubated for 12 hours. Then, the quartz was rinsed in PBS (Sigma) and was saturated with BSA (Sigma) solution (1 % in mass) for one hour. At the end, the quartz was rinsed in PBS and then mounted into the cell. The same protocol was kept for the other biospecies : goat antibodies (Immunotech) against rabbit IgG (Jackson Immunoresearch Labs).

Biological reagents

The corresponding “antigen” (peroxidase and rabbit IgG) solution circulated above the crystal, which allowed a direct binding of the analyte to the fixed antibodies.

Results

The biosensor specificity was checked for each detected antigen. Figure 7A presents a preliminary control, for rabbit IgG detection, where a pseudo selective layer was made only by BSA adsorption onto the gold electrode : the rabbit IgG interaction did not occurr because no frequency shift was observed even with concentrated solutions of rabbit IgG (25 µg ml^{-1}) (figure 7A). Thus, in this case, they was no non-specific interaction. Then, a complementary test was performed as it is shown in figure 7B : the bioselective layer was built as it was described just before; goat IgG were immobilized directly onto the gold electrode and saturated with BSA. An other antigen, here alkaline phosphatase, flew over the QCM : as previously, a steady microbalance frequency signal was observed, which indicated that non-spe-

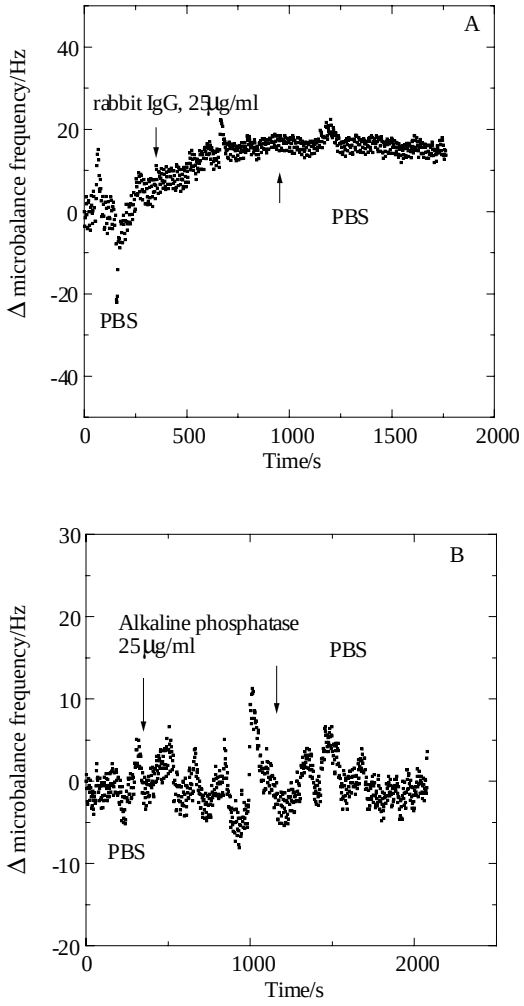


Figure 7. Specificity test for rabbit IgG detection : A) gold electrode coated with BSA and B) gold electrode coated with goat IgG (antibody) saturated with BSA.

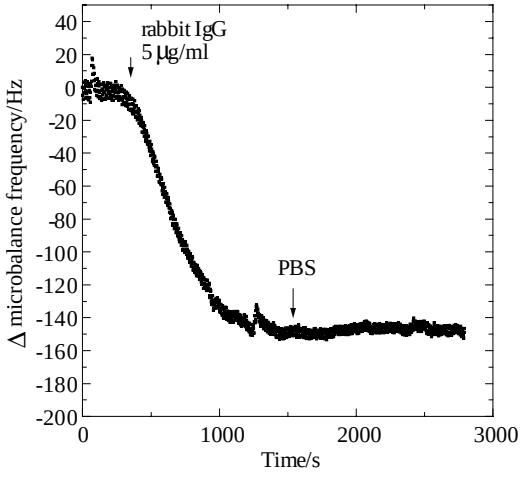


Figure 8. QCM response to addition of rabbit IgG ("antigen").

cific interactions occur. It proves the good quality of this layer and the good specificity of the antigen/antibody couple used in this experiment.

In a second step, an attempt of direct detection was performed as it is presented in figure 8. For a 5 µg ml^{-1} rabbit IgG solution injected in the QCM cell, a large decrease of the microbalance frequency, Δf = 150 Hz, was observed. In a second phase, pure PBS solution was injected : the microbalance frequency remained constant. This observation led to two conclusions : on the one hand, the interactions between antibodies (goat IgG) against antigens (rabbit IgG) was strong as the frequency did not change and on the other hand, changes due to solution viscosity/density were negligible as the measurable signal was constant when the nature of the solution was modified. Moreover, 90 % of the signal was obtained very quickly, the time response being around a few minutes.

The same approach was performed with an another antigen/antibody couple : peroxidase/anti peroxidase. The first step was focused on the biolayer specificity : figure 9 presents the antigen response for a QCM coated only with adsorbed BSA. The same conclusion can be drawn, as for the previous device : this BSA layer is insensitive to the interaction with peroxidase (PO). Secondly, direct detection was tested : a PO solution flowed over the microbalance surface and the frequency decreased consecutively to the interaction. Therefore, antigen detection was feasible thanks to the high sensitivity of the transducer used here. This sensitivity was sufficient to directly detect the binding event between small biomolecules, such as peroxidase, and the specific antibody layer (figure 10). The 27 MHz microbalance allowed this direct transduction to be carried out without the realization of a sandwich assay.

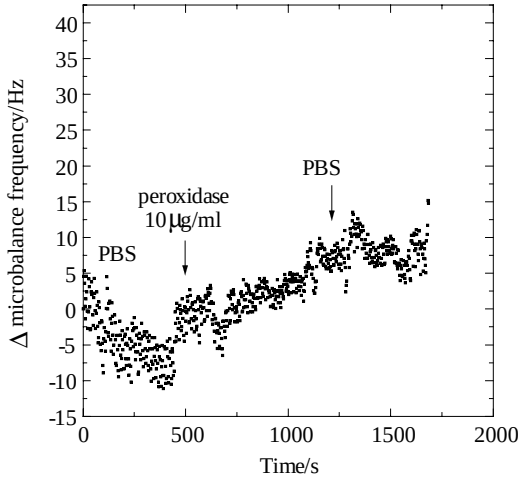


Figure 9. Specificity test for PO detection; gold electrode is coated only with BSA.

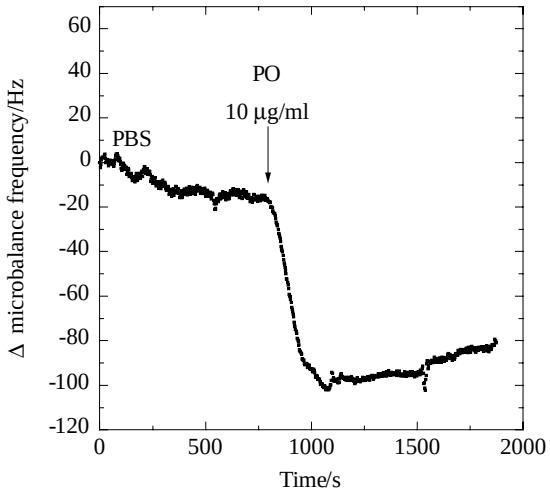


Figure 10. Detection of PO with a 27 MHz device.

Potentialities for the development of biosensors

In this paragraph our discussion will be focused only on classical QCM devices used for biosensing in the immunosensing field or for DNA hybridization studies.

Shons et *al*. [44] in 1972 were the first to described an immunosensor based on a QCM device. BSA was immobilized onto a crystal previously coated with Nyebar C solution which led to an hydrophobic surface able to held BSA. First, the resonant frequency was measured in air, then the prepared device was dipped in a BSA solution following by a drying and a new measurement in air (F_1). Secondly, the device was tested in a solution containing antibodies anti-BSA. After rinsing, the quartz was dried and the resonant frequency was measured. The frequency shift determined in air ($\Delta F = F_1-F_2$) was proportional to the antibody concentration. This technique was called "dip and dry" and was currently used after due to the facility of used for the resonator. Then, in 1989 [49], direct measurements were performed in liquid. Table III presents different applications using QCM for immunosensing.

Tableau III. Examples of immunosensors based on QCM piezoelectric transducers.

Authors	*Year*	*Resonance frequency (MHz)*	*Measurement technique*	*Analyte to be detected*	*Bioselective layer*	*Test*	*Immobilization technique*
Muramatsu et *al.* [48]	1986	9	dip and dry	*Candida albicans* in PBS	antibodies anti C. albicans	direct	silanization and chemical grafting with glutaraldehyde
Davis and Leary [49]	1989	10	continuous	Ig G	antibodies anti Ig G	direct	adsorption of protein A then antibodies
Guilbault et *al.* [39]	1992	10	dip and dry	atrazine in water	antibodies anti atrazine	direct	– silanization and glutaraldehyde – adsorption of protein A then Ig
Kößlinger et *al.* [50]	1992	20	continuous	antibodies anti HIV	Synthetic HIV peptide	direct	adsorption of peptide et saturation with gelatine
Chu et *al.* [37]	1995	10	dip and dry	Human IgM	Sheep antibodies anti human IgM	direct	(HEMA/MMA) polymer then covalentgrafting with CNBr
Steegborn and Skadal [38]	1997	10	continuous	atrazine in water	atrazine coupled to BSA	competitive	silanization and covalent grafting of l'atrazine-BSA with glutaraldehyde
Harteveld [51]	1997	20	Continuous	SEB	Antibodies anti SEB	competitive	Adsorption
Mascini [52]	1998	10	Continuous	Anti BSA HIgG	BSA Anti human IgG	direct	Adsorption Covalent on SAM Dextran

An another attractive potentialities for QCM concerns the DNA hybridization studies where very recent publications presents interesting results : for studying the kinetics of hybridization [45], following the methodology for DNA immobilization [46] and testing high sensitive devices at 27 MHz [47].

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