

## QUARTZ CRYSTAL MICROBALANCE (QCM) USED IN CHEMICAL AND BIOLOGICAL APPLICATIONS IN REAL TIME

Gh.V. CIMPOCA<sup>1,2</sup>, I. V. POPESCU<sup>1,2</sup>

**Abstract:** *In this paper we develop and characterize sensors and applications with a quartz crystal microbalance (QCM). The sensitivity, selectivity, reproducibility and detection limit of QCM sensors have also been discussed. Quartz Crystals are used in Microbalances (QCM) as sensors to determine mass changes as a result of frequency changes and becoming into a good alternative analytical method in a great deal of applications such as biosensors, analysis of biomolecular interactions, study of bacterial adhesion at specific interfaces, pathogen and microorganism detection, study of polymer film-biomolecule or cell-substrate interactions, immunosensors and extensive use in fluids and polymer characterization. In general, the following steps are involved in sensor applications: (a) measurement of the appropriate sensors parameters; (b) extraction of the corresponding physical parameters related to the model selected for the specific application, starting from the measurements in the previous step; (c) interpretation of the physical, chemical and biological phenomena which enable to explain the extracted parameters of the selected model.*

**Keywords:** Quartz Crystal Microbalance, Newtonian liquids, solid/fluid interfaces

### 1. Introduction

The high sensitivity and the real-time monitoring of mass changes on the sensor crystal make Quartz Crystal Microbalance (QCM) a very attractive technique for a large range of applications [1]. Especially, the development of QCM systems for use in fluids or with viscoelastic deposits has dramatically increased the interest towards this technique. Major advantages of the QCM technique used for liquid systems are that it allows a label-free detection of molecules. The QCM is capable of measuring mass changes as small as a fraction of a monolayer or single layer of atoms.

QCM crystals becoming into a good alternative analytical method in a great deal of applications such as biosensors, analysis of biomolecular interactions, study of bacterial adhesion at specific interfaces, pathogen and microorganism detection, study of polymer film-biomolecule or cell-substrate interactions, immunosensors and extensive use in fluids and polymer characterization and electrochemical applications among others [2-8].

<sup>1</sup>Professor at Valahia University of Targoviste, Academy of Romanian Scientists, 54 Splaiul Independenței, Bucharest, RO-050094, [valcimpoca@yahoo.com](mailto:valcimpoca@yahoo.com)

<sup>2</sup>Academy of Romanian Scientists, 54 Splaiul Independenței, Bucharest, RO-050094, [ivpopes@yahoo.com](mailto:ivpopes@yahoo.com)

Measurement of the appropriate sensors parameters is very important because an erroneous sensor parameter characterization can lead to the misinterpretation of the phenomena involved during the experiment. This problem occurs in thickness shear mode (TSM) microbalance sensors, when significant changes in the acoustic load or in parallel capacitance of the sensor occur during the experiment, if appropriate electronic interfaces are not used. These effects are accentuated in the case of heavy acoustic loads and particularly happen oscillators are used as QCM drivers [2, 3]. Also, in research environment, the most common QCM crystal applications include metal deposition monitors, chemical reaction monitors, biomedical sensors, and environmental monitoring applications etc. Other applications include detection of mass, density, viscosity, adsorption, desorption, and corrosion [1, 4-6].

Eventually, the QCM was used also in gaseous environments, e.g. as gas and humidity sensors and for the detection of aerosols [8]. The ability to provide real-time monitoring of chemical contaminants in water samples can be used for a variety of applications: on-line monitoring of contaminants in process, recycle, and waste water; groundwater quality monitoring; detection of contaminants in streams, lakes and water supplies; monitoring dumping in off-shore waterways [9] QCM studies of adsorption from aqueous solution are one example of this type of approach. In these cases, it is almost always appropriate to assume that the system is homogeneous along the crystal surface. In this paper, we are interested in the opposite situation, where spatial heterogeneities exist primarily in the lateral direction. In particular, it is study a spreading contact area of one material surrounded by another at the QCM surface [10, 16].

## 2. Theory, Operation and Calibration

Sauerbrey was the first to recognize the potential usefulness of the Quartz Crystal Microbalance (QCM) technology and demonstrate the extremely sensitive nature of these piezoelectric devices towards mass changes at the surface of QCM electrodes. The results of his work are embodied in the Sauerbrey equation [7], which relates the mass change per unit area at the QCM electrode surface to the observed change in oscillation frequency of the crystal:

$$\Delta f = -C_f \times \Delta m \quad (1)$$

Where  $\Delta f$  = the observed frequency change in Hz,  $\Delta m$  = the change in mass per unit area in  $\text{g/cm}^2$ ,  $C_f$  = the sensitivity factor for the crystal ( $56.6 \text{ Hz } \mu\text{g}^{-1} \text{ cm}^2$  for a 5 MHz AT-cut quartz crystal at room temperature).

The equation does not apply to non-Newtonian fluids, viscoelastic films, thick films, and liquid environment. For a 5 MHz quartz crystal, with  $\Delta f=1 \text{ Hz}$  and sensor area  $1 \text{ cm}^2$ , result  $\Delta m= 17.7 \text{ ng}$ .

The Sauerbrey equation relies on a linear sensitivity factor,  $C_f$ , which is a fundamental property of the QCM crystal. Thus, in theory, the QCM mass sensor does not require calibration. However, it must be kept in mind, that the Sauerbrey equation is only strictly applicable to uniform, rigid, thin-film deposits<sup>2</sup>. Vacuum and gas phase thin-film depositions which fail to fulfill any of these conditions actually exhibit more complicated frequency-mass correlations and often require some calibration to yield accurate results. For many years, QCMs were regarded as just gas-phase mass detectors, however; recently their application has been extended since scientists realized that they can be operated in contact with liquids and viscoelastic deposits. In this case, both resonance frequency and series resonance resistance of the quartz oscillator are important to completely characterize the material in contact with the crystal electrode. The development of QCM systems for use in fluids opened a new world of applications, including electrochemistry and microrheology.

More recent developments have focused on tailoring electrode surface chemistry (i.e. specialized polymer coatings) so that these devices can be applied as discriminating mass detectors for (1) specific gas detection, (2) environmental monitoring, (3) biosensing and (4) basic surface-molecule interaction studies. When the QCM comes in contact with a solution, there is a decrease in frequency that is dependent upon the viscosity and the density of the solution. A quantitative understanding of the resonator behavior is a prerequisite for proper interpretation of experimental results under total liquid immersion. This problem was first treated by Glassford, and later by Kanazawa and Gordon [7]. Kanazawa's treatment of the influence of the solution properties on the crystal (equation 2) permits the prediction of the change in resonance frequency which accompanies immersion of the crystal into a viscous medium:

$$\Delta f = -f_U^{1/2} \left( \frac{\rho_L \eta_L}{\pi \rho_q \mu_q} \right)^{1/2} \quad (2)$$

where  $f_U$  = frequency of oscillation of unloaded crystal,  $\rho_q$  = density of quartz =  $2.648 \text{ g cm}^{-3}$ ,  $\mu_q$  = shear modulus of quartz =  $2.947 \times 10^{11} \text{ g cm}^{-1} \text{ s}^{-2}$ ,  $\rho_L$  = density of the liquid in contact with the electrode,  $\eta_L$  = viscosity of the liquid in contact with the electrode. Viscous coupling of the liquid medium to the oscillating crystal surface results not only in a decrease in the series resonant frequency but also in damping of the resonant oscillation - the viscous loss is manifested as an increase in series resonance resistance,  $R$ , of the QCM resonator. Thus,  $R$  serves as an excellent independent measure of viscous loading by the medium (liquid or soft-film) at the crystal's surface.  $\Delta f$  and  $\Delta R$  measurements are both routinely used as independent indicators of mass loading and viscosity at the crystal-liquid interface of the QCM resonator during chemical and electrochemical depositions in solution.

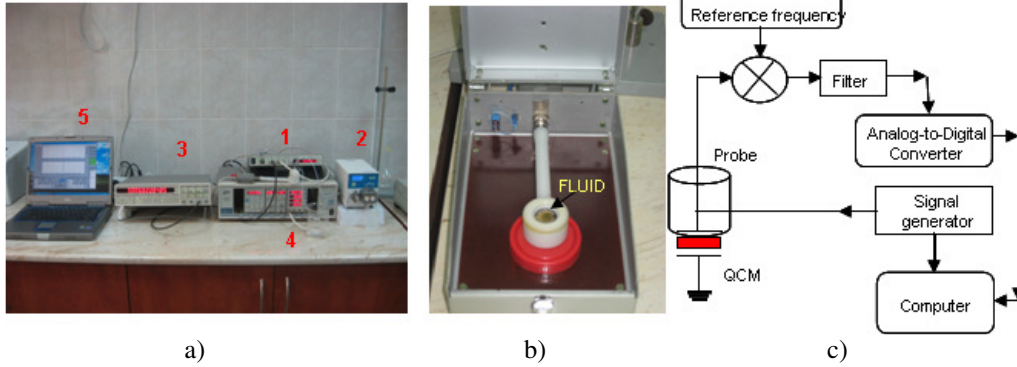
In a separate study, a Butterworth-Van Dyke equivalent circuit model was applied to derive a linear relationship between the change in series resonance resistance,  $\Delta R$ , of the quartz oscillator and  $(\rho_L \eta_L)^{1/2}$  under liquid loading:

$$\Delta R = \left( \frac{n \omega_s L_u}{\pi} \right) \left( \frac{2 \omega_s \rho_L \eta_L}{\rho_q \eta_q} \right)^{1/2} \quad (3)$$

Where:  $\Delta R$  = change in series resonance resistance in  $\Omega$ ,  $n$  = number of sides in contact with liquid,  $\omega_s$  = angular frequency at series resonance ( $2\pi f_s$ ),  $L_u$  = inductance for the unperturbed (dry) resonator, usually in mH.

### 3. QCM Systems

The standard QCM System (from Stanford Research Systems-SRS) is a stand-alone instrument with a built-in frequency counter, resistance meter, personal computer and printer (figure 1).

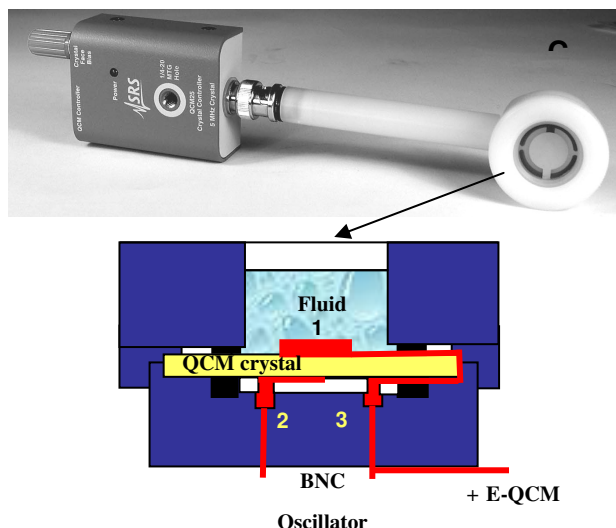


**Fig.1.** a) QCM System; b) Quartz crystal holder and c) Diagram for operation.

It includes controller, crystal oscillator electronics, crystal holder, and quartz crystals [18]. Series resonance frequency and resistance are measured and displayed directly on the front panel, and there is an analog output proportional to the relative frequency to interface directly with potentiostats (E-QCM applications). The QCM may be locked to a user supplied 10 MHz clock for ultra-stable frequency measurements. In addition, the QCM has an RS-232 interface and comes with both Windows and LabVIEW software providing real-time display, analysis and storage of our QCM data. Supplementary, we have micro fluidic pump; Ultra-stable Rubidium Frequency Standard with 2ppb stability and 0.0001 Hz sensibilities. The ultra precise temperature meter measure “*in situ*” temperature, between 4 K and 700 K with 0.1 K.

#### 3.1. The layout and the fabrication of the QCM crystal

The layout of the sensors for our systems must to connect at Crystal Holder cavity with wrap around electrode pointing towards reference pin (Figure 2).



**Fig. 2.** Crystal Holder cavity with quartz crystal sensor.

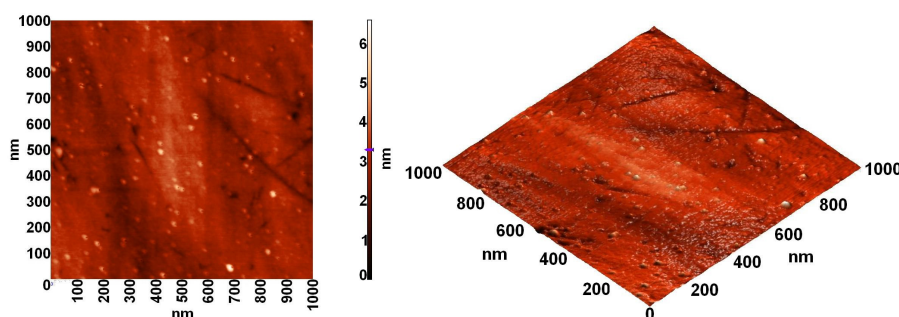
Figure 3 shows a top and bottom view of a standard 1 inch diameter QCM Sensor Crystal, with what is called a “wrap-around” electrode configuration. A conductive metallic pad is used to allow connection to the front electrode (liquid surface) from the back of the crystal (electrical contact surface). This is required for mounting of the crystal into its Crystal Holder and for proper operation in conductive liquids.



**Fig. 3.** Quartz crystal sensors with gold electrode.

The exposed area of the front electrode in contact with the liquid is  $\sim 1.37 \text{ cm}^2$ , however, the active electrode oscillation (i.e. displacement area) is mostly restricted to the area of overlap between the two circular pad electrodes or  $\sim 0.40 \text{ cm}^2$ . All standard 1 inch QCM sensor crystals are planar, with both faces parallel to within  $1 \mu\text{m}$ .

Crystal thickness is about 330  $\mu\text{m}$  as required for fundamental oscillation at 5 MHz. The large diameter of the crystal (1 inch), and the relatively much smaller oscillation area, assures good separation between the active electrode pads and the mounting structure of the holder and minimizes the coupling of other resonant modes to the thickness shear oscillation. Electrode surface roughness can cause large apparent mass loadings due to the liquid that is trapped within pores at the crystal surface. As a general rule, optically polished crystals (50  $\text{\AA}$  average surface roughnesses) are required to obtain good agreement between theory and experiment during liquid immersion experiments. In our experience we use crystal from Stanford Research Systems-SRS and from IMT-Bucharest, special designed for QCM System. In the figure 4 we represent the images AFM of surface roughness for quartz crystals in 2D and 3D.



**Fig.4.** The 2D and 3D AFM images for quartz crystal without metallization.

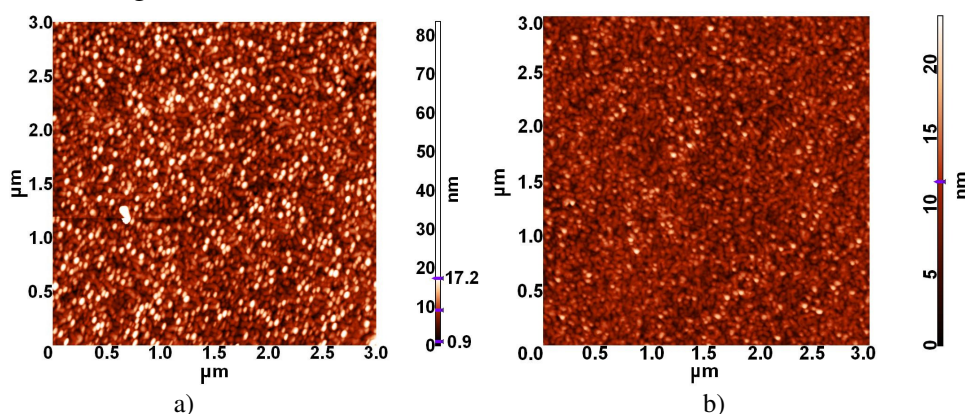
Water Immersion Test usually provides a quick-and-easy way to test the quality of a crystal finish for liquid-QCM by way of measurement frequency and resistance. These measurements shall be performed in the pure water and are independent of the electrodes (type of metal deposition).

Materials for electrodes can be: metals, inorganic oxides films and polymers films or other materials.

- Metals: Gold (Au); Aluminum (Al); Carbon (C); Chromium (Cr); Cobalt (Co); Copper (Cu); Molybdenum (Mo); Nickel (Ni); Palladium (Pd); Platinum (Pt); Silicon (Si); Silver (Ag); Titanium (Ti); Tungsten (W); Zinc (Zn); Stainless steel; etc..
- Inorganic oxides films:  $\text{SiO}/\text{SiO}_2$ ,  $\text{SiO}_2$ ,  $\text{TiO}/\text{TiO}_2$ ,  $\text{SnO}_2$ ; ITO ( $\text{InO}_2$  and  $\text{SnO}_2$ ).;etc.
- Polymer: PANi, Teflon AF, PMMA, PS, PC, PE, PP, AKD, etc.

Inorganic oxides and Polymers can be deposited on the metallic electrodes such as continuum film or with dimensions in the nano and sub-micrometer region. The latter constitute “Hollow particles” (nanocontainers) with dimensions in the nano and sub-micrometer region which possess great potential for encapsulation of well-defined quantities and of large sized host molecules into their empty core

domains. Gold electrodes (100-1000 nm thick) are the most commonly used in QCM applications because of the ease with which gold is evaporated. A thin (20-50 nm) adhesion layer of Cr, Ti or Si is often deposited directly on the quartz to aid in the adhesion of the metal electrode. Chromium is recommended for polished blank surfaces and also for crystals operated in a harsh environment. However, Cr can be a problem in some electrochemical applications - spurious electrochemical responses can sometimes result if diffusion of the adhesion material to the electrode surface occurs. In that case Titanium is recommended as a substitute adhesive layer. If Ti is still a problem, Palladium can be utilized as a barrier metal between the Cr or Ti and the primary metallization. A zinc layer sandwiched between the adhesion layer and the electrode pad on the front or deposition side of the crystal has a relatively low bulk modulus, and has been shown to absorb stress imposed by the deposited materials. The buffer layer prolongs useful crystal life, particularly when depositing dielectric materials. The crystal should have a modest surface roughness as keying-in structure. The buffer layer can be a zinc layer of 600 nm sandwiched between a titanium or chromium adhesion layer of 10 to 16 nm and a gold, silver, or aluminum outer electrode layer of about 200 nm. For applications requiring transparent electrodes, tin-doped indium oxide (ITO) electrodes are recommended. ITO is routinely used to make transparent conductive coatings which are applied in a wide variety of applications. Transparent electrodes are often used in cell adhesion studies when visualization of the sample cells is required during the experiments. In principle, any type of material that can be deposited onto the surface of the underlying metal electrode can be used on the sensor crystals. All metallization was deposition by Sputtering in Ar atmosphere. Images AFM with CrAu and TiAu metallization are show in the figure 5.



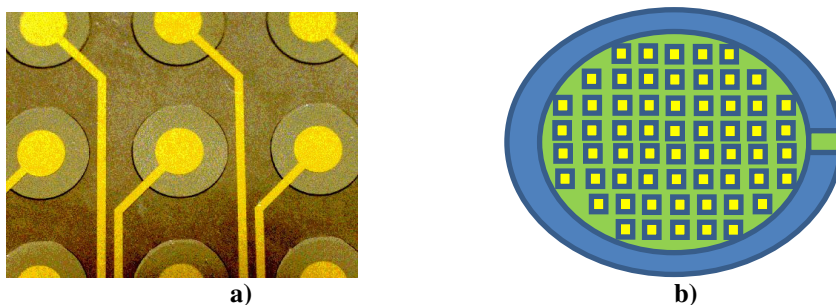
**Fig. 5.** AFM on a) CrAu/quartz substrate and b) TiAu/Quartz substrate.

The only limitation on the use of such materials is that their deposition must be carried out in such a way that the temperature of the quartz crystal does not exceed 573 °C, above which  $\alpha$ -quartz loses its piezoelectric activity.



For our experiences we use three materials for electrode: 1) CrAu (20 nm Cr and 200 nm Au); 2) TiAu (50 nm Ti and 200 nm Au) and 3) TiPt (50 A Ti and 200 nm Pt). It is important to keep in mind that mass changes at the QCM active electrode surface influence the frequency because these electrodes are part of the composite resonator composed of (1) the quartz crystal, (2) its metal electrodes, (3) any film deposited on the electrodes and (4) any liquid adjacent to the electrode surface which experiences shear forces. For this reason, when electrodes become delaminated (due to poor adhesion of the under layer or stress caused by the deposit), discontinuous changes in frequency occur which render the crystal useless. The system is compatible with other types of sensors, depending on the application.

Figure 6 represent two types of sensors: a) multiple sensors with micrometer-sized and b) a sensor with metallization windows in the dielectric films.



**Fig. 6.** Sensors for QCM:

a) multiple micro sensor and b) sensor with nano windows in dielectric films.

### 3.2. Mass Sensitivity and Loads

The quartz crystal microbalance is an extremely sensitive sensor capable of measuring mass changes in the nanogram/cm<sup>2</sup> range with a wide dynamic range extending into the 100 µg/cm<sup>2</sup> range. The minimum detectable mass change is typically a few ng/cm<sup>2</sup> and limited by the noise specifications of the crystal oscillator and the resolution of the frequency counter used to measure frequency shifts. For example, based on Sauerbrey's equation (1), a minimum detectable mass specification of 0.18ng/cm<sup>2</sup> should be expected from a QCM system with a 0.01 Hz frequency measurement resolution. As a comparison, consider that a monolayer of a 20 kDa protein (100% coverage) adsorbed on a QCM crystal amounts to roughly 10 Hz frequency shift, or 180ng/cm<sup>2</sup>. Larger changes in frequency with temperature should be expected when QCM crystals are immersed in liquids. This is due to the coupling of the shear mode oscillation with the temperature dependent viscosity and density of the fluid. The temperature coefficient due to this effect is ~8 Hz/°C (and 4 Ω/°C) for water. As a result, for experiments in liquid phase in which the frequency is to be monitored at length, the temperature must be controlled to at least 0.1°C, and preferably better. If temperature control is not possible or practical, it is always good practice to wait at



least 30 minutes before performing any accurate measurements after the crystal comes in contact with a new liquid medium and measure the temperature of the solution around the crystal during the experiments to perform temperature compensation. QCM sensor crystals can be coated with virtually any material as long as it can be deposited in a sufficiently thin, uniform and well-attached layer. Layer thicknesses typically vary from a few Angstrom to a few micrometers. The maximum layer thickness depends on the viscoelastic properties of the coating material. As a general rule, thicker layers are possible for more rigid coating materials.

The maximum load on a crystal is limited by two factors: (1) total damping or (2) lost sensitivity. For highly viscous or solid materials, the damping of the crystal increases with increasing layer thickness. At a certain thickness (usually a couple of micrometers) the damping becomes so high that the crystal can no longer be driven, i.e. the measurement fails due to lack of oscillation. More elastic materials do not couple completely to the crystal's oscillation. With increasing layer thickness the outermost parts of the attached layer will couple weakly and at a certain thickness (usually a few micrometers) it is lost completely. Oscillation is still detected, and a frequency is still measured, but the equipment can only sense the part of the layer in the vicinity of the crystal.

### **3.3. Techniques of use with QCM**

Quartz crystal microbalance can be used in several ways, depending on the application [19-24]. Thus it can be used with a static or dynamic fluid; with a fixed oscillation frequency oscillation or interruptions to relax fluid. QCM also can work as a stand-alone system or coupled with other measurement systems, examples being thing in coupled with electrochemical measuring instruments or coupled with the AFM.

#### **3.3.1. QCM standard with the static fluid**

In this technique the fluid is put in the Crystal Holder cavity with crystal such as in figure 2. We have all time the control about temperature. The quantity of fluid is between 0.3 ml and maximum 1 ml. The best quantity of fluid is 0.5 ml. Kanazawa's treatment of the influence of the solution properties on the crystal (equation 2 and 3) permits the prediction of the change in resonance frequency and resistance which accompanies immersion of the crystal into a viscous medium. Measurements are exhausted solution in time before and the time between two measurements is programmed from 0.1 seconds to 10 seconds. The total time of measurement depends on the viscosity of the solution and can be from 1 hour to 24 hours.

#### **3.3.2. QCM with dynamic fluids (QCM-F)**

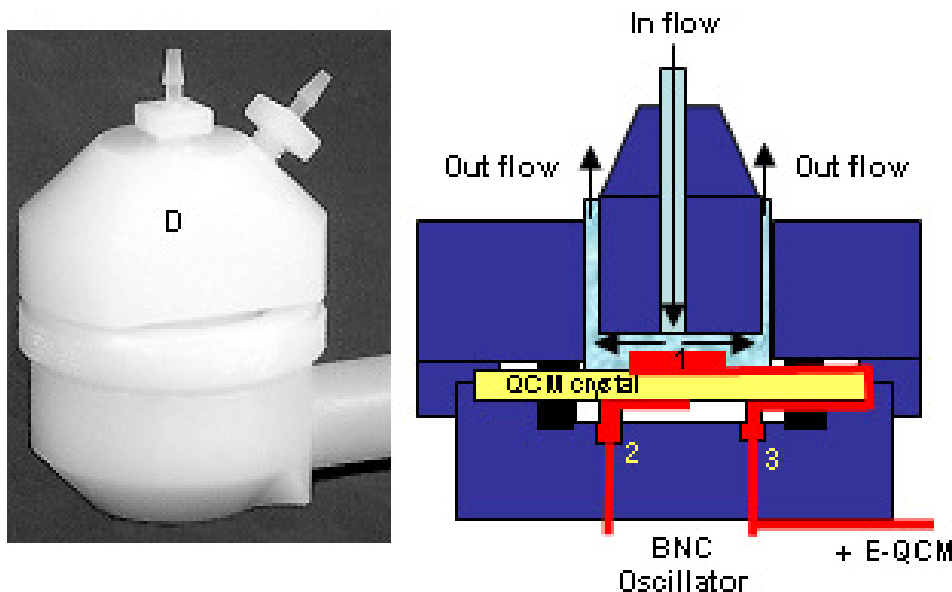
Other QCM method for fluids analysis is with the Flow Cell Adapter attached to the crystal holder where we create a small volume Stagnation Point Flow Cell. Use the Axial Flow Cell Adapter (figure 7) for adsorption/desorption studies

when: (1) well defined flow conditions are needed, (2) high sensitivity is required, (3) small rinse volume is desirable and (4) flow-induced surface shear forces need to be minimized. In the axial flow cell the sample flows radially outward from the input port at the center of the cell to the exit channel at the edge of the cell, in a volume of about 150  $\mu\text{l}$ . The sample solution is perpendicularly injected towards the flat surface of the QCM crystal.

The stagnation point is located at the center of the crystal electrode, overlapping the area of highest sensitivity of the flat QCM oscillator. The stagnation point flow cell was specifically designed for the study of adsorption kinetics of particles, micelles and chemical and biological molecules at solid-liquid interfaces, under well-controlled hydrodynamic conditions.

Examples of surface-volume processes that have been studied with the aid of stagnation flow cells include: DNA protein interactions, protein adsorption, cell adhesion, bio fouling, biocompatibility, polymer degradation and hydration, biofilm formation, self-assembled monolayer, etc.

A quartz crystal microbalance with flowing (QCM-F) technique has been employed to examine the detergency process involved when solid organic soils are removed from hard surfaces by surfactants.



**Fig.7.** - Schematic representation of the liquid flow pattern in the Axial Flow Cell.

Experiments have been designed to focus on real time monitoring of the physicochemical aspects of detergency. The introduction of using the QCM for detecting adsorption effects and for monitoring the differences in corrosion due to the adsorption of a Volatile Corrosion Inhibitors (VCI) is of recent interest.

The QCM in recent investigations appear to have some definite credibility in detecting the adsorption of a few VCIs.

It is a widely known fact that certain chemicals are very effective at emitting a vapor that can molecularly adsorb onto a metals surface.

This molecular adsorption usually consists of a few monolayers and a few studies have been published on the adsorption of VCIs

### 3.3.3. QCM-D Technology

Quartz Crystal Microbalance with Dissipation monitoring, QCM-D, is a patented technique that senses variations in the natural resonance characteristics of a thin slice of quartz when changes occur at the surface such as protein adsorption.

The differences in the oscillation characteristics of the sensor are tracked as changes to the surface occur and measured as changes in resonance frequency and dissipation.

Q-Sense has a range of instrumentation and software tools to allow sophisticated interpretation of the sensing data.

Mass, thickness, viscosity and elasticity of adsorbed layers, plotted versus time, are typical output data of QCM-D measurements. QCM-D has the ability to track events such as adsorption and interaction (fig. 8).

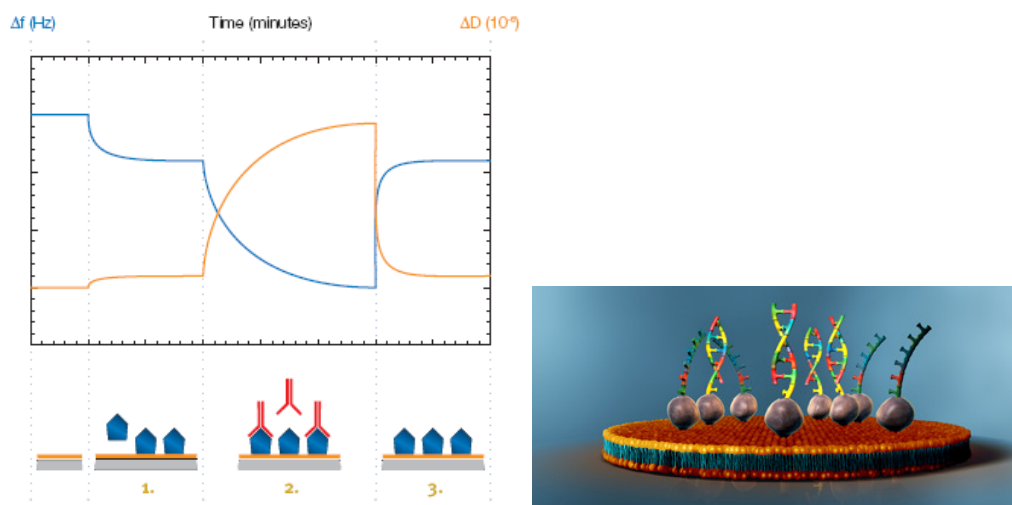
1. In this example, the binding of a globular molecule to the surface is seen with a moderate frequency response,  $\Delta f$ , but low dissipation,  $\Delta D$ .
2. When a more elongated molecule binds on top of the first molecular layer, the QCM-D response changes resulting in higher frequency and much higher dissipation levels.  
This indicates that water has been coupled resulting in a layer that is softer and less rigid than the first layer.
3. Following rinsing, where the elongated molecule is removed, the frequency and dissipation reduce again.

Q-Tools software provides analysis based on viscoelastic models and kinetics calculations.

Using the data gained, the software can calculate thickness, viscosity and elasticity. This is possible for soft films that do not obey the linear relation between change in frequency and change in mass.

Many applications of QCM-D are in medicine researches.

For example QCM-D has been used in the study of functionalized surfaces, developed to allow specific interactions studies such as DNA hybridization.



**Fig. 8.** Adsorption and interaction of molecules determined by QCM-D.

As the functionalized surface is built up, QCM-D follows in detail how molecules form such as, for example, whether lipid vesicles form a bilayer. The information gathered by QCM-D has proved important in the design of sophisticated surface modifications for bio-recognition studies.

In the case of non-rigid over layers it is obvious that the full capacity of the QCM is not exploited by the conventional measurement of the frequency shifts alone.

The most obvious quantity to measure, in addition to the resonant frequency,  $f$ , is the damping of the crystal oscillation, i.e. its dissipation factor,  $D$  (inversely proportional to the  $Q$  factor).

$D$  is the sum of the various energy dissipating subsystems in the composite oscillator ( $D_{\text{tot}} = \sum D_i$ ) and can, therefore, reveal the dissipative properties of viscoelastic over layers.

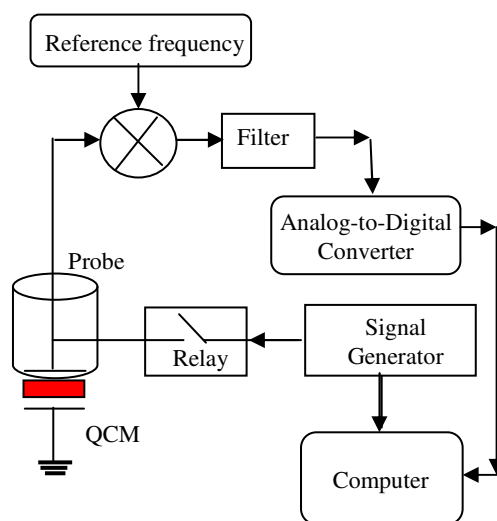
The  $f$  and  $D$  measurements were performed using a prototype system. The parameter  $D$  is calculated with the formula 4:

$$D = E_{\text{lost}} / 2\pi E_{\text{stored}} \quad (4)$$

where  $E_{\text{lost}}$  is the energy lost during one oscillation cycle and  $E_{\text{stored}}$  is the total energy stored in the oscillator (order of magnitude is  $10^{-6}$ ).

Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) is a real-time, nanoscale technique for analyzing surface phenomena including thin film formation, interactions and reactions.

The operation of all instruments is essentially the same and is shown schematically in Fig. 9.



**Fig. 9.** QCM-D Experimental System.

The measurement is based on the principle that, when the driving power to a piezoelectric crystal oscillator is switched off, the voltage over (or the current through) the crystal decays as an exponentially damped sinusoidal. By recording the voltage (or current) over the crystal during the decay, and numerically fitting this curve to an exponentially damped sinusoidal, both the resonant frequency and the dissipation factor of the crystal can be obtained simultaneously. Prototype had the ability to excite the crystal at its fundamental or the third overtone, whereas the other two could only excite the crystal at its fundamental resonant frequency. The  $f$  and  $D$  sampling rate was 1 Hz (when  $f$  and  $D$  were measured at both the fundamental and third overtone, the sampling rate was halved). We used polished 5 MHz crystals. The QCM liquid chamber was temperature stabilized to  $\pm 0.1$  °C to avoid drifts in  $f$  and  $D$ . The measurements were performed in a stagnant liquid cell, designed to provide a fast, no perturbing exchange of the liquid over one side of the QCM sensor

### 3.3.4. E-QCM (Electrochemical QCM)

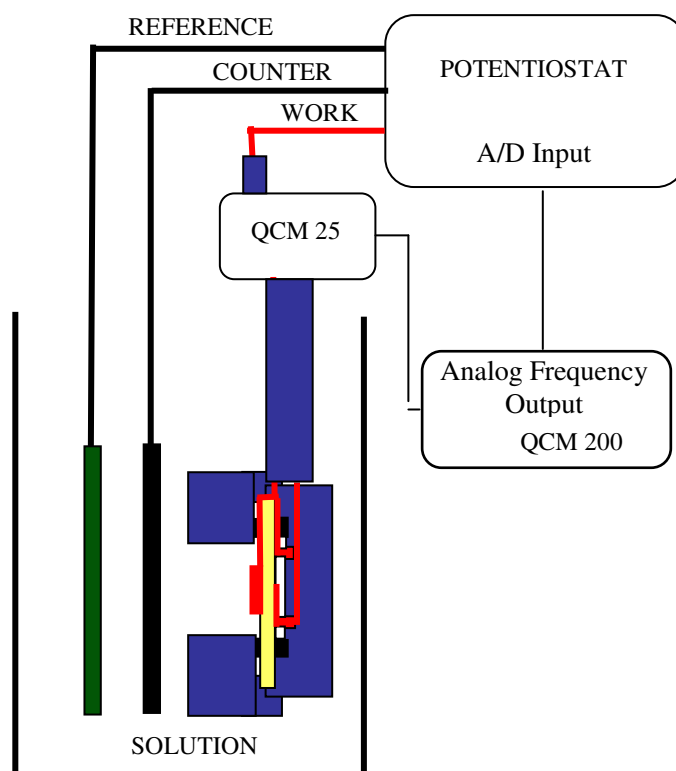
In most electrochemical experiments, mass changes occur as material is deposited or lost from the “working” electrode. It is of interest to monitor those changes simultaneously with the electrochemical response, and the E-QCM is the standard means of doing so. As a gravimetric probe, the E-QCM has been used in many types of electrochemical studies, including: under potential deposition of metals, corrosion, oxide formation, dissolution studies, adsorption/desorption of surfactants and changes in conductive polymer films during redox processes. The E-QCM has been extensively used to study polymer modified electrodes, particularly as a gravimetric tool to follow redox processes. However, the polymer over layer must exhibit no changes in rigidity during the electrochemical process.

Otherwise, the viscoelastic changes will also contribute to the frequency change, leading to an erroneous interpretation of the mass changes. As a consequence, it is essential to determine whether or not viscoelastic properties of the polymer film influence the frequency measurement during polymer film experiments.

A schematic diagram of the apparatus for electrochemical quartz crystal microbalance (E-QCM) experiments is given in Figure 10.

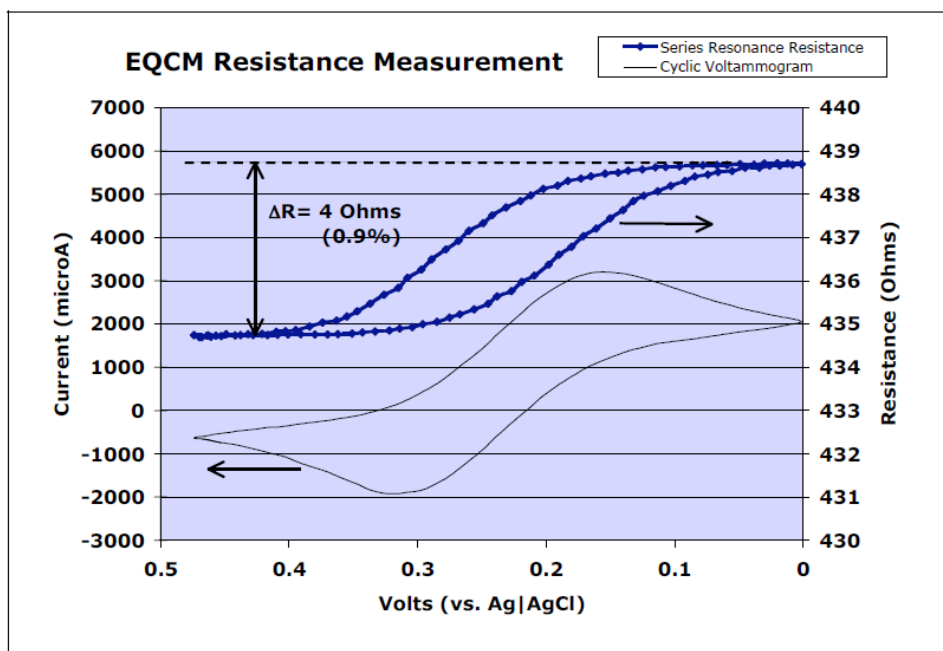
In this example, a 1 inch diameter polished QCM crystal is mounted on a Crystal Holder with only one electrode exposed to the conductive solution. The Crystal Holder is connected to a QCM25 Crystal Oscillator, and the liquid-contact electrode is connected to the “working electrode” lead of the potentiostat (through the Crystal Face Bias connector of the QCM25 Crystal Oscillator).

In figure 11 we represent changes in series resonance resistance at the E-QCM sensor during the electrochemical oxidation/reduction of  $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ . The resistance–potential diagram shows a resistance increase in perfect synchronization with the generation of a viscous layer of  $\text{Fe}(\text{CN})_6^{4-}$  (reduction) adjacent to the QCM electrode.



**Fig. 10.** E-QCM system.



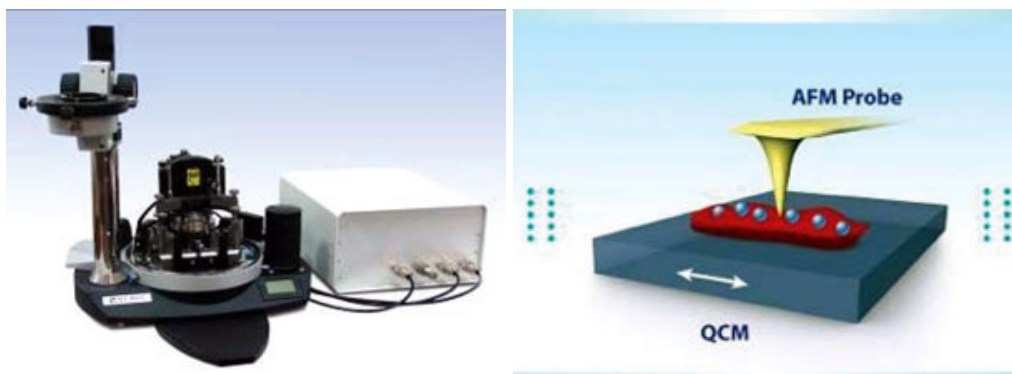


**Fig. 11.** Changes in series resonance resistance at the E-QCM sensor during the electrochemical oxidation/reduction of  $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ .

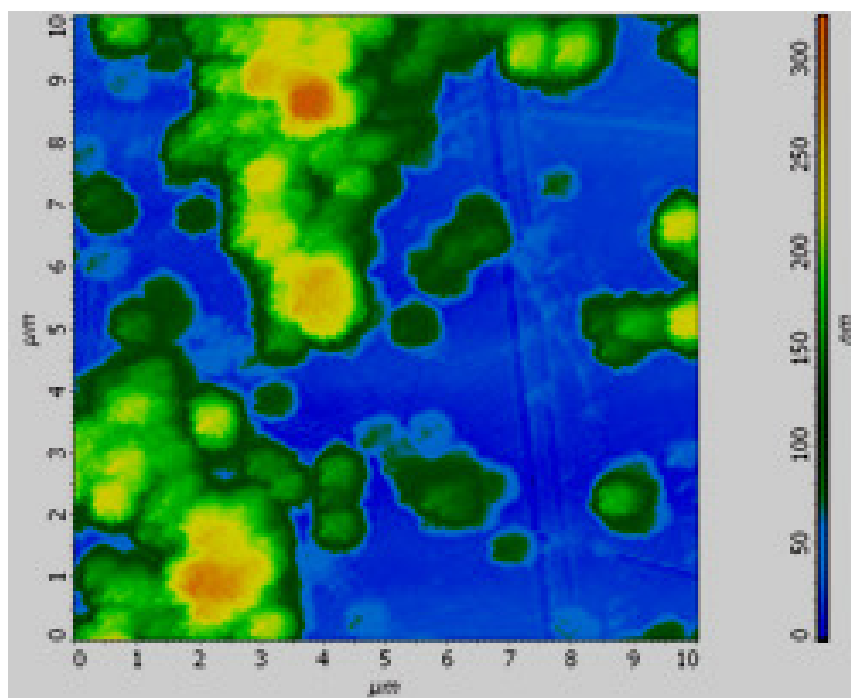
The resistance–potential diagram shows a resistance increase in perfect synchronization with the generation of a viscous layer of  $\text{Fe}(\text{CN})_6^{4-}$  (reduction) adjacent to the QCM electrode.

### 3.3.5. QCM combined with AFM

The combined QCM/AFM instrument provides simultaneous in-situ observation of the samples topography (up to nm) and change in mass (up to 0.3 ng). So the researchers get more data for comprehensive interpretation from a single platform. Integra platform coupled AFM with QCM (figure 12).



**Fig. 12.** QCM + AFM = enhanced capabilities for a single platform (INTEGRA).



**Fig. 13.** AFM on quartz sensor for QCM (CrAu metal deposition).

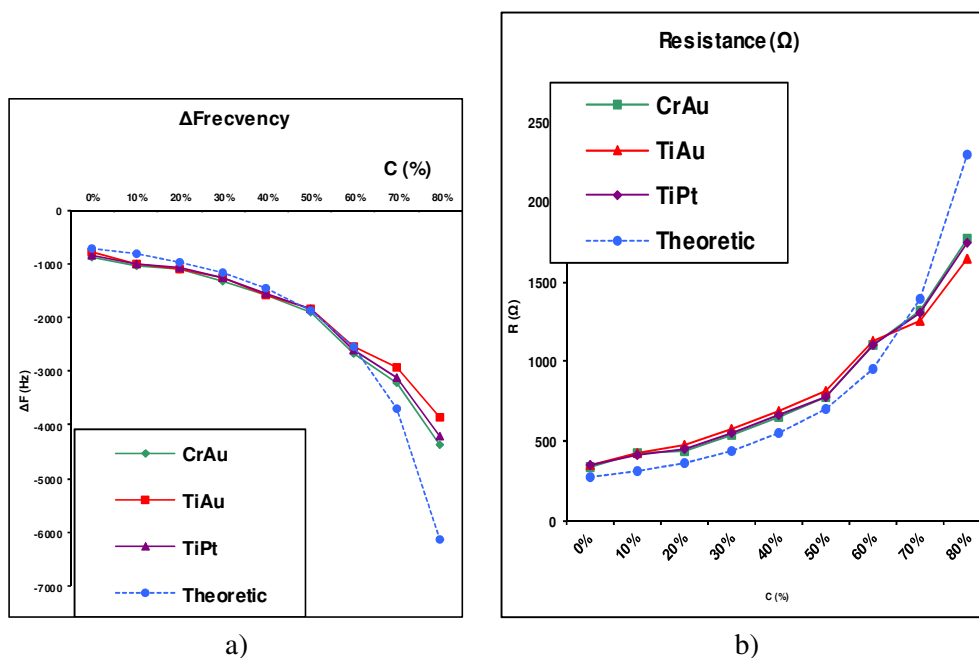
Basic frequency of oscillation with the deposited substance: 5,007,536.3 Hz  
 Quartz frequency stability on 5 minutes interval: about  $\pm 0.06$  Hz

#### 4. Results and discussions

QCM uses techniques that are easy to use, but requires more work. Not being a technical homologated measurement results should be compared to other techniques. Each liquid shows a definite imprint. From this point of view it must be fingerprinted standard liquid or known. For each type of measurements are calibrations of sensors measuring device used. Are prepared comprehensive database which then helps us to identify the fluid being measured.

##### 4.1. Calibration

The QCM System was specifically designed for operation under heavy viscous loading. The standard Crystal Holder supports operation in gas and liquid environments, and provides single-electrode exposure to as required for compatibility with electrochemical QCM measurements. The Crystal Oscillator provides both frequency and resistance change signals and will maintain oscillation up to a series resonance resistance of about 10 k $\Omega$  (it will support crystal operation in highly viscous solutions up to 88% glycerol in water. The tight correspondence between theory and experiment is clearly illustrated by figure 14.



**Fig. 14.** a) Frequency Shift (Hz) and b) Resistance

(□ versus glycerol weight percentage (in water) as predicted by theory and as measured with QCM and a 5 MHz polished crystal with CrAu; TiAu and TiPt Metallization.

A comparison against the theoretical predictions of equations 1 and 2 is usually a good starting point when testing a new experimental liquid QCM setup. Two common “checkup” procedures are described. In a water immersion, for a metal coated, 5 MHz, polished crystal, and assuming that the electrode capacitance has been properly cancelled, a decrease in frequency of about 715 Hz, and an increase in series resonance resistance of about 380  $\Omega$ , is expected when switching from air to complete immersion in water at 20°C. In a Glycerol/H<sub>2</sub>O immersion the frequency shift and resistance shift depends on the concentration of glycerol and aims equations 1 and 2.

We observe a little deviation from the formulas [25], because that glycerol is inhomogeneous after 70% in water.

Operation at increasing glycerol concentrations is an excellent test of a QCM experimental setup, and should provide predictable results up to more than 88% glycerol.

In procedures, an agreement between measured and expected values within  $\pm 2.5\%$  is generally considered acceptable for glycerol concentrations up to 70%.

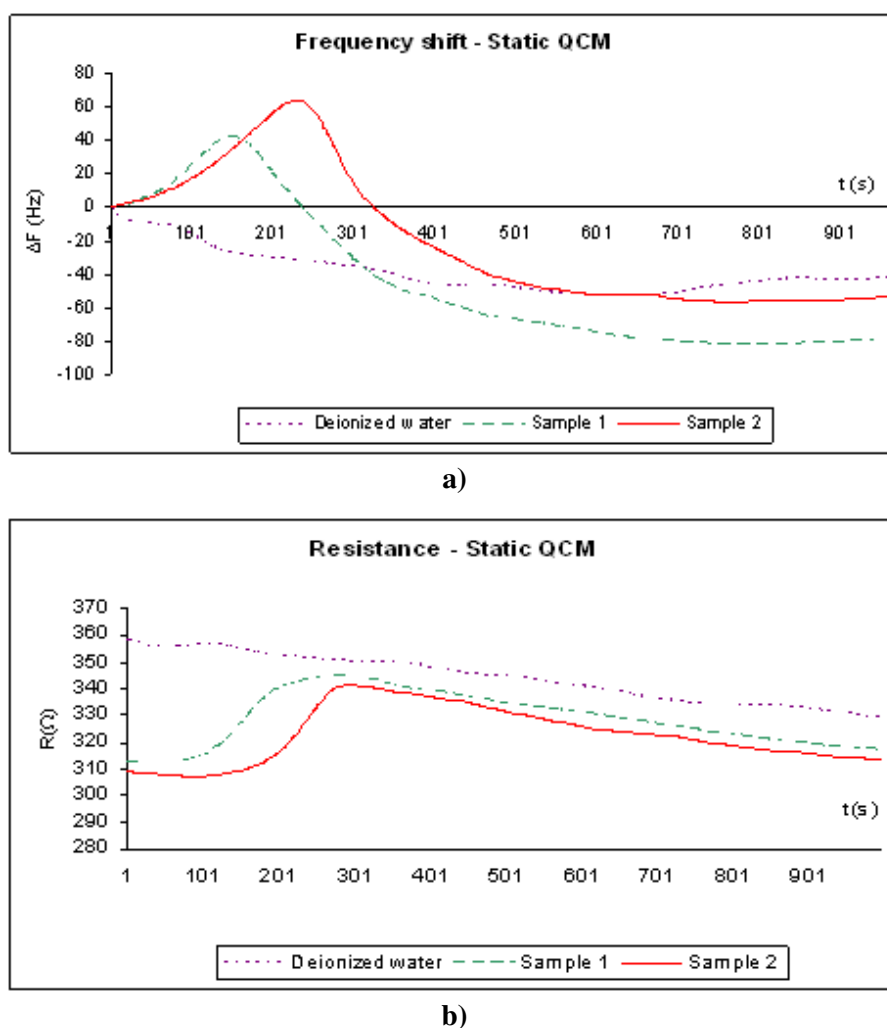
Frequency shifts are much larger (at least a factor of two) and unpredictable for unpolished crystals so they are not recommended for system checkup.

## 4.2. Water Quality

The ability to provide real-time monitoring of chemical contaminants in water samples can be used for a variety of applications: on-line monitoring of contaminants in process, recycle, and waste water; groundwater quality monitoring; detection of contaminants in streams, lakes and water supplies; monitoring dumping in off-shore waterways [18;26-31].

In our experience we use three types of water: deionised water; drinking water from town Targoviste (sample 1) and groundwater from countryside (sample 2).

The results from QCM static are shown in figure 15.



**Fig. 15.** Data for QCM static: frequency shift (a) and resistance (b) versus time for deionized water, drinking water (sample 1) and groundwater (sample 2).

Figure 15 show that in the pure water (deionized water) adsorption of molecule practically missing when in the sample 1 and sample 2 the adsorptions are present.

The results are in concordance with the other analytic methods:

Atomic Absorption Spectroscopy (AAS): conductivity/resistivity;

Total Dissolved Solid (TDA), pH and salinity.

In the Table 1 and the Table 2 we present the results after these methods.

**Table 1.** The results after AAS of waters analyses  
for determination the metal contaminates

	Cd (µg/ml)	Cr (µg/ml)	Cu (µg/ml)	Fe (µg/ml)	Ni (µg/ml)	Pb (µg/ml)	Se (µg/ml)	Zn (µg/ml)
Sample 1	0.001	0.513	0	0	0	0.022	1.256	0.006
Sample 2	0.001	0.433	0	0	0	0.054	1.155	0.004
Sample 1 after QCM	0	0.313	0	0	0	0.031	0.214	0
Sample 2 after QCM	0.001	0.579	0	0	0	0.033	0.322	0.003

**Table 2.** The results after pH; Conductivity; Salinity; and TDS  
for water analyses at 25 °C

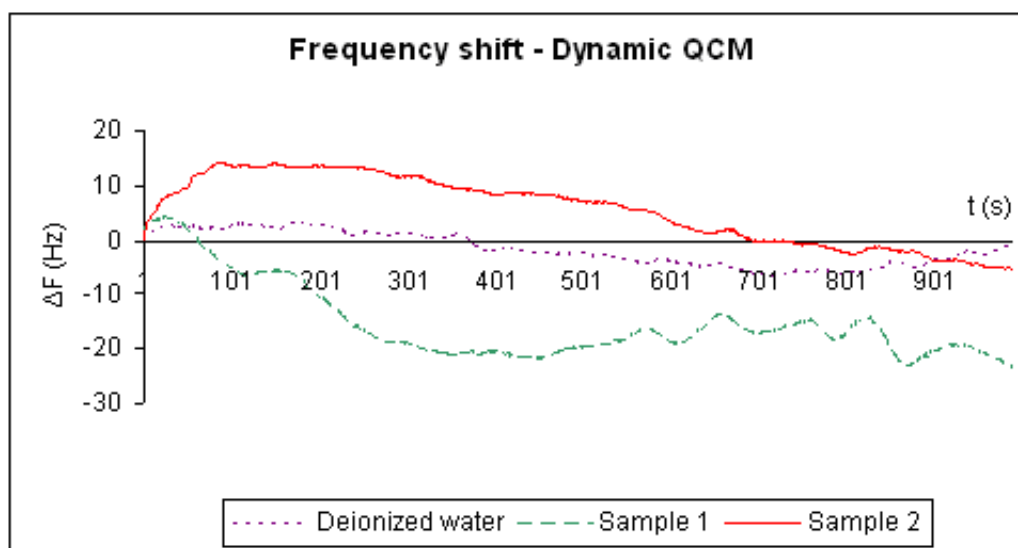
	pH	Conductivity µS/m	Salinity ‰	Total Dissolved Solid mg/l	Temperature °C
Sample 2	7.02	629	0.3	301	25
Sample 3	7.35	1237	0.6	601	25

Other experimental method is when we use standard Flow Injection Analysis (FIA) and Sequential Injection Analysis (SIA) equipment which is compatible with QCM flow cell operation.

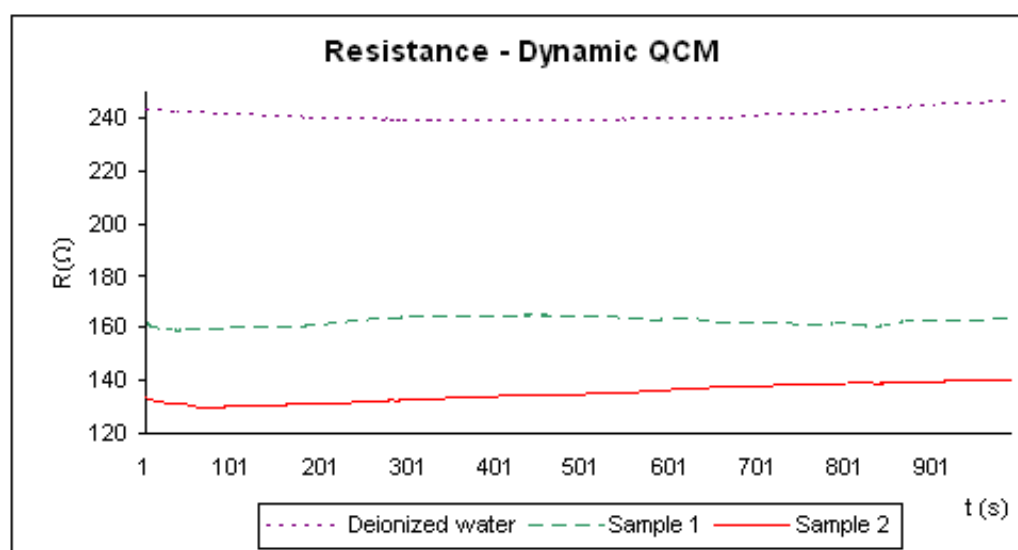
The fluid debit is adjustable between 0.01 ml/minute and 10 ml/minute. Optimum is between 0.5 ml/minute and 1 ml/minute.

The maximum debit (10 ml/minute) is for washing after each solution.

The results for water quality are presented in the figure 16.



a)



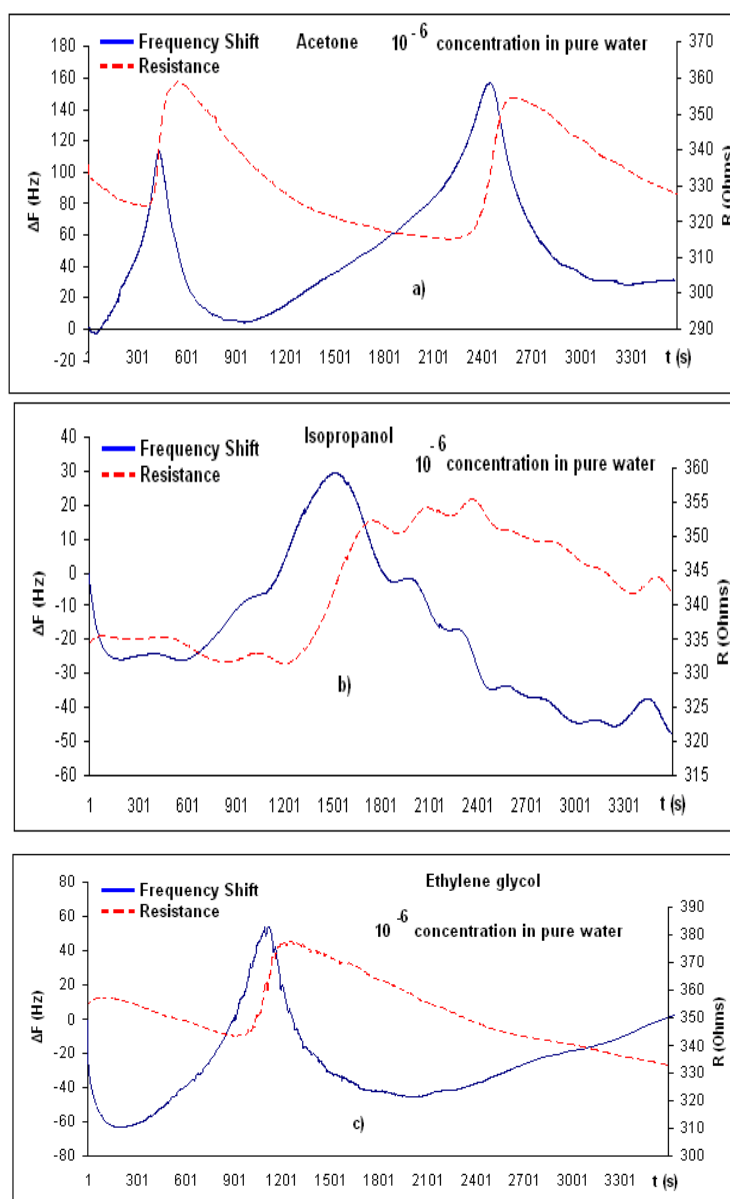
b)

**Fig. 16.** Data for QCM dynamic:  
frequency shift (a) and resistance (b) versus time for deionized water,  
drinking water (sample 1) and groundwater (sample 2)

One thing for sure is that, in these waters which were measure, we did not detect traces of volatile organic compounds (VOC).

As we shall see in the figure 17, the characteristics of frequency shift and resistance for any trace of volatile organic compounds are specifics.





**Fig. 17.** Data from QCM: frequency shift and resistance for volatile organic compounds (VOC) in water: a) Acetone in pure water (1mg/L or 1ppm); b) Isopropanol in pure water (1mg/L or 1 ppm) and c) Ethylene glycol in pure water (1mg/L or 1ppm).

For our experiences we prepared pure water with 1 part per million (ppm) of VOC: acetone, isopropanol and ethylene glycol. In the figure 17a it can see that the variations of frequency shift and resistance of acetone are cyclical processes of adsorption/desorption. The variation of frequency shift are very high, 120 Hz or about 2088 ng in mass.

Also, and for isopropanol and ethylene glycol, the variations for frequency shift are very high, over 100 Hz, or 1740 ng in mass desorption/adsorption. Characteristic for each substance and concentration for traces VOC in water are the diagrams: frequency shift and resistance versus time, which are unique, as fingerprints. Adsorption of standard volatile components water: acetone, isopropanol and ethylene glycol and uncoated quartz crystal surfaces was measured as a function of time. Results indicated that nature of the coating greatly influences total adsorbed masses, which was attributed to the binding specificity between coatings and volatile compounds.

#### 4.3. Traces of Potassium Cyanide in water

One of the most poisonous ions from drinking water is cyanide. This ion is an extremely poisonous compound commonly used in industries such as precious metals mining, metal plating shops, steel mills, and plastic and fertilizer factories. The various industries worldwide produce as much as 1400000 tons of toxic cyanide per year. Cyanide's toxicity results from its propensity to bind to the iron in cytochrome *c* oxidase, interfering with electron transport and resulting in hypoxia.

Since it is lethal to human beings as well as aquatic life, the U.S. EPA regulates cyanide content at the very low levels of 0.2 and 0.005 mg/L for drinking water and environmental primary standards, respectively [32]. The current detection methods include ion chromatography, headspace gas chromatography analyses, spectrophotometric determinations and other methods.

These detection methods suffer from a number of disadvantages such as requiring large sample sizes, long analysis times, high detection limits, and poor precision, largely due to interferences. The current methods are also labor intensive and require significant special skill and training. The focus of our current research is to provide a single analytical sensor that combines the advantages found in many of the previously mentioned cyanide studies [33-35].

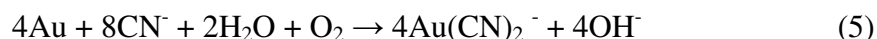
The developed detection system provides rapid cyanide determinations with little sample preparation or instrument supervision. The high sensitivity and the real-time monitoring of mass changes on the sensor crystal make Quartz Crystal Microbalance (QCM) a very attractive technique this application. The quartz crystal microbalance is an extremely sensitive sensor capable of measuring mass changes in the nanogram/cm<sup>2</sup> range with a wide dynamic range extending into the 100 µg/cm<sup>2</sup> range.

The minimum detectable mass change is typically a few ng/cm<sup>2</sup> and limited by the noise specifications of the crystal oscillator and the resolution of the frequency counter used to measure frequency shifts. The sensibility depends of the detector area and the type of solid electrode in contact with the fluids.

H. Sun and others tried to determine cyanide trace levels in water using Piezoelectric Quartz Crystal (PQC) with photochemical deposited nano-sized Ag particles on electrode [34]. Our method is easier and more stable, because we use CrAu metallization for metal electrode in contact with solution [38].

The QCM is capable of measuring mass changes as small as a fraction of a monolayer or single layer of atoms. We show that QCM is a good alternative analytical method in a great deal of applications such as analysis of molecular interactions.

The presence of cyanide in solution results in the selective formation of a soluble dicyano-gold complex according to the Elsner reaction 5:



The resulting loss of gold from the electrode is detected by the piezoelectric crystal as a resonant frequency change  $\Delta f$  and resistivity change. In your paper, Y.G. Timofeyenko et al, from Department of Chemistry, Idaho State University, USA, present a cyanide detection method suitable for drinking and fresh water, which offers many operational advantages that should allow it to be a widely accepted routine monitoring system [36].

Also, J.J. Rosentreter and all in the US patent present the real-time measurement of aqueous cyanide [37].

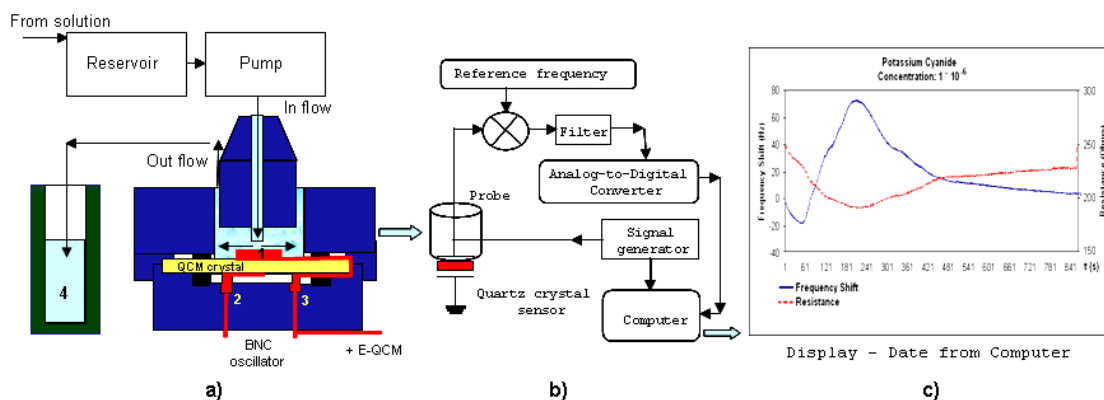
In our paper we present two methods for cyanide trace in water, based on QCM systems: with static fluids and dynamic fluids [38].

In both cases we present the characteristics of frequency shift and resistance in real-time. For each concentration of cyanide in water we obtain specific fingerprints and specific values of frequency shift and resistance.

### **Experimental system QCM for cyanide**

The QCM System (from Stanford Research Systems-SRS) is a stand-alone instrument with a built-in frequency counter, resistance meter, flow pump, special flow cell and accessories, personal computer and printer (figure 18). It includes controller, crystal oscillator electronics, crystal holder, and quartz crystals. Series resonance frequency and resistance are measured and displayed directly on the front panel.

In addition, the QCM has an RS-232 interface and comes with both Windows and LabVIEW software providing real-time display, analysis and storage of our QCM data. Supplementary, we have Ultra-stable Rubidium Frequency Standard with 2 ppb stability and 0.0001 Hz sensibilities and the ultraprecise temperature meter measure “*in situ*” temperature, between 4 K and 700 K with 0.1 K.



**Fig. 18.** QCM System: a) Schematic Diagram of flux by system  
b) Electronic diagram c) Data graphics.

We can measure the parameters of solutions both static and dynamic using a pump with adjustable flow from 0.01 mL/minute to 10 mL/minute. The maximum flow is for to clean cell and to remove previous fluid.

After this step the flow is kept constant for measurement of parameters.

The flows used were between 0.3 mL/minute and 1 mL/minute. If the flow is null after cleaning we can measure in the static condition.

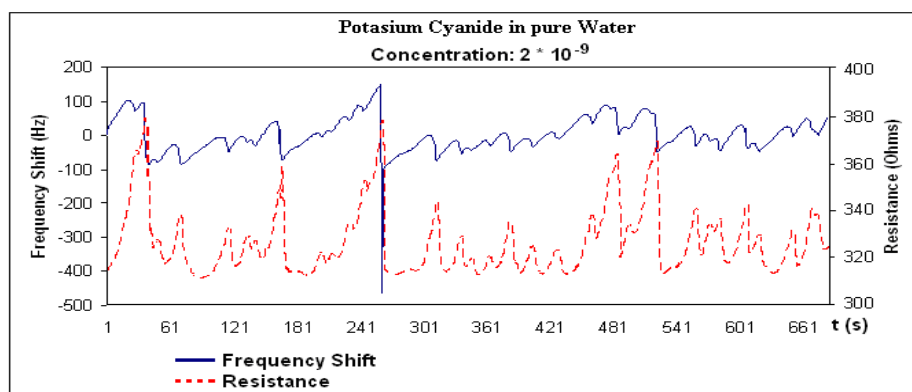
Use the Axial Flow Cell Adapter for adsorption/desorption studies when: (1) well defined flow conditions are needed, (2) high sensitivity is required, (3) small rinse volume is desirable and (4) flow-induced surface shear forces need to be minimized. Under static conditions (the flow is zero) and the sample applies direct on quartz sensor of cell from QCM system. The quantity of solution is 1 mL for each sample.

For dynamic the flows used were: 0.2 mL/minute; 0.5 mL/minute; 0.7 mL/minute and 1 mL/minute. All chemicals used were of the highest grade commercially available. Standard cyanide solutions were prepared using 0.01 g of CN/L of potassium cyanide stock solution, adjusted to pH 12 with potassium hydroxide for safe handling. All solutions were stored under light and heat protected conditions. Dilute solutions were made with pure water (deionized water with resistivity minimum 18.2 MΩ) and adjusted to pH 12 with potassium hydroxide. Increased sample pH provides higher sensitivity.

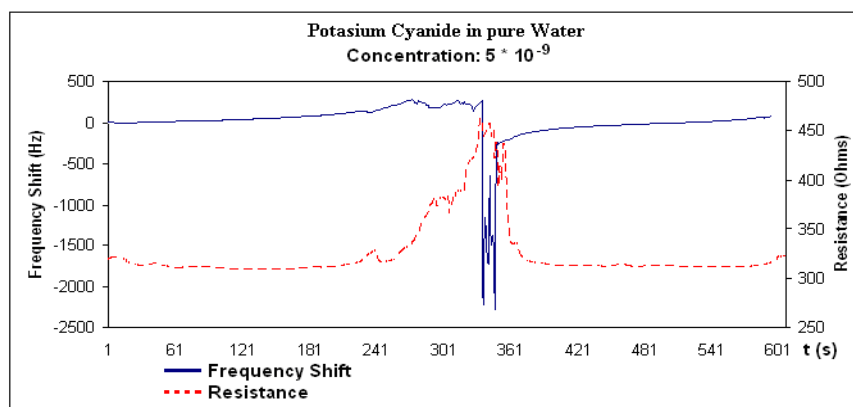
### Results and discussions for cyanide

In the figure 19, 20 and 21 we present the results after analysis with QCM static for 2 ppb, 5 ppb and 1 ppm of cyanide in water with 12 pH.

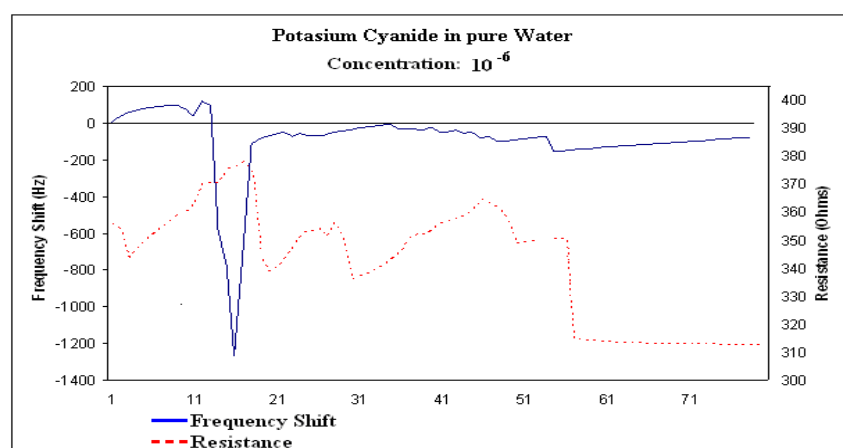
The quantity of liquid use on the quartz crystal in these figures is 1mL. For other concentration and other quantities of liquid we obtain other graphics.



**Fig. 19.** Frequency Shift and resistance versus time with QCM static for 2 ppb concentration Potassium Cyanide in pure water.



**Fig. 20.** Frequency Shift and resistance versus time with QCM static for 5ppb concentration Potassium Cyanide in pure water.



**Fig. 21.** Frequency Shift and resistance versus time with QCM static for 1ppm concentration Potassium Cyanide in pure water.

At concentrations below 5 ppb of cyanide in water, the adsorption processes alternates with desorption processes at short intervals, frequency shift is  $\pm 100$  Hz average and at a time (about 260 s) jump to about 500 Hz.

Cyanide ions in the first moments are adsorbed on the gold electrode where reacts with gold, in concordance with Elsner reaction. Every two cyanide ions will be issued a gold atom. Because at 1 mL solution there is a very small number of cyanide ions, they are consumed quickly (about 4 minutes).

For concentrations of cyanide higher, frequency shift jump to about 2000 Hz after 6 minute for 5 ppb and 20 seconds for 6 ppm. Also for these concentration cyanide ions are limited in number.

To increase sensitivity and to have a larger number of cyanide ions we can use dynamic fluids. We can measure the parameters of cyanide using a pump with adjustable flow. The maximum flow is for to clean cell and to remove previous fluid. After this step the flow is kept constant for measurement of parameters.

The sample preparation involves dissolution of potassium cyanide (KCN) in distilled water at different concentrations such as 0; 0.2; 0.5; 1 and 2 ppm. The 9 and 12 pH values of the samples were corrected with KOH (20%). It is chosen these values of pH for study because for the pH values 9 and 12 was obtained a good repeatability in according with literature data.

In the figures 22 and 23 are represented the data from QCM-F with dynamic fluid for cyanide in pure water with 2 ppb and 5 ppb concentrations.

The flow is constant at 0.5 mL/minute. If a concentration of 2 ppb frequency shift is very low (about 3 Hz after 10 minutes) and presents short instability (with variations from positive to negative), for 5 ppb frequency shift is continuous positive (mass is negative) and after 10 minutes the value of its is 12 Hz.

In the figure 7 we show the results after QCM-F dynamic, with 1 ml/minute debit, 1ppm concentration Potassium Cyanide in pure water with 12 pH.

For our standard crystal frequency response is 45 Hz/ppm of 1ppm cyanide in solution, per 1 minute of aspiration (figure 24).

This response is increased to 90 Hz/ppm in 2 min of aspiration.

This increase in detection as a function of increased analysis times is a consequence of the direct relationship between dissolution of the crystal's coating according to the Elsner reaction and the crystal's vibration frequency.

Increased detection times allow for a greater number of cyanide ions to come in contact with the gold electrode, thereby resulting in a greater loss of mass and greater increase in frequency according to the Elsner equation.



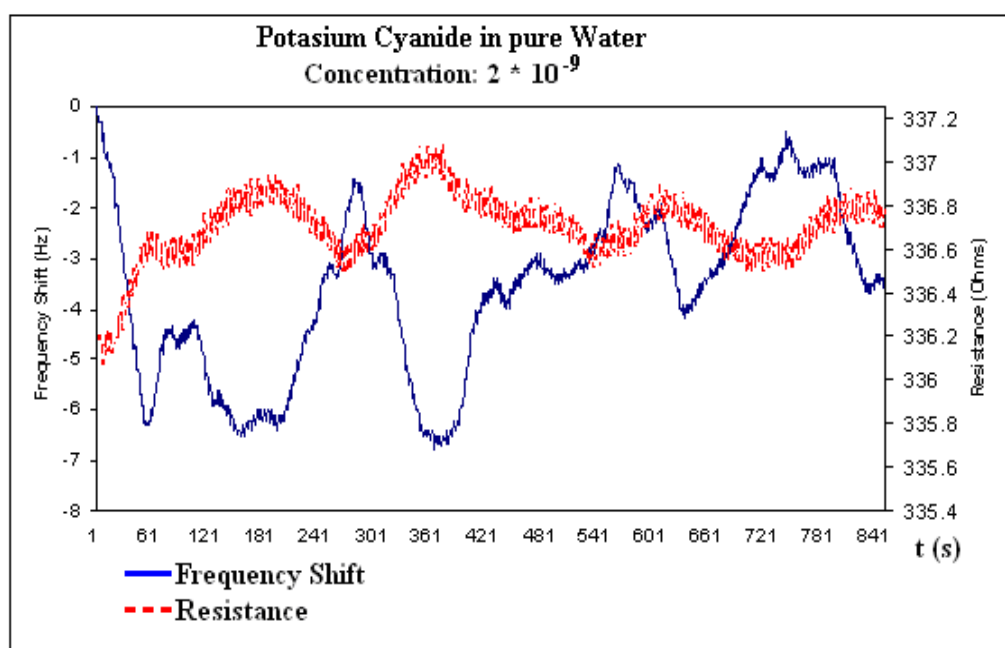


Fig. 22. Frequency Shift and resistance versus time with QCM-F dynamic, 1mL/minute debit in Potassium Cyanide (2ppb concentration).

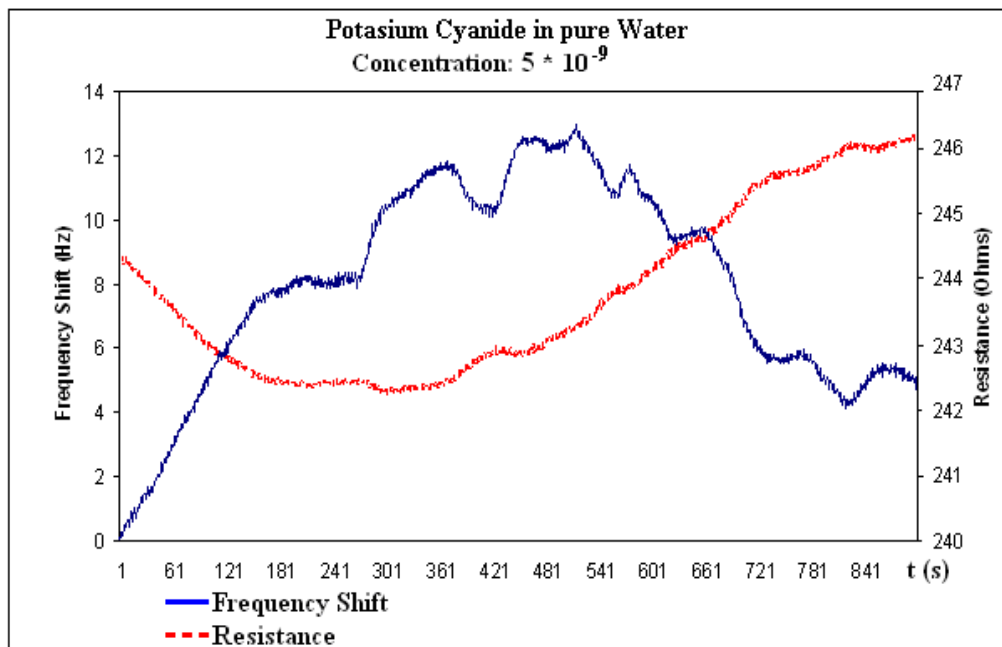
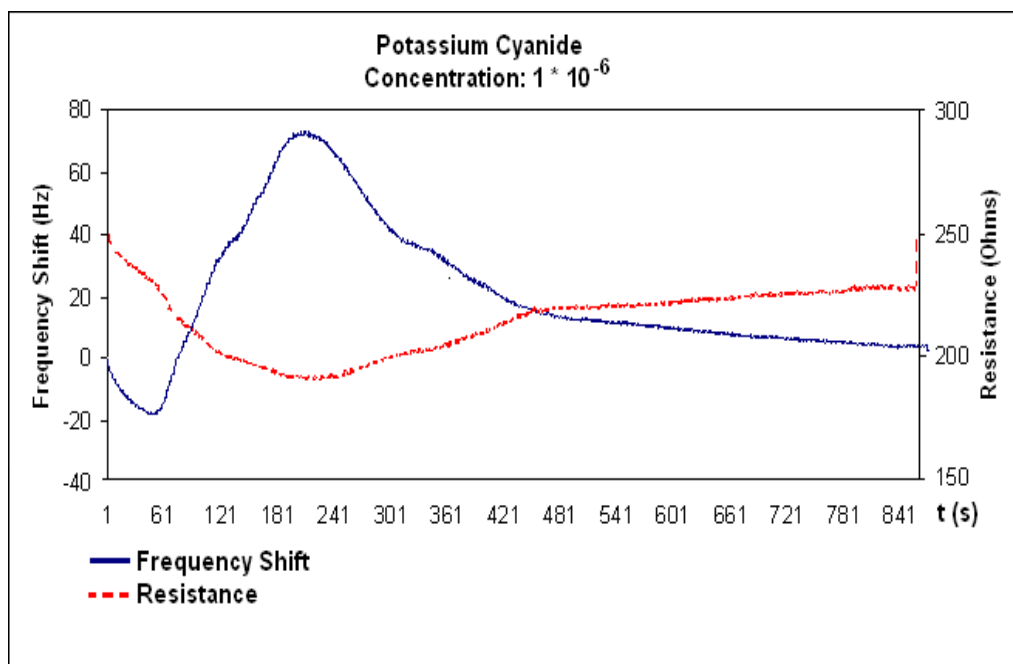


Fig. 23. Frequency Shift and resistance versus time with QCM-F dynamic, 1mL/minute debit in Potassium Cyanide (5ppb concentration).



**Fig. 24.** Frequency Shift and resistance versus time with QCM-F dynamic, 1 mL/minute debit in Potassium Cyanide (1ppm concentration).

### Verification and confirmation method

The method proposed by us can be verified by two independent procedures. The first is the analysis of solution after QCM testing with Atomic Absorption Spectroscopy (AAS). According to Elsner reaction we need find traces of gold in solution, almost half the concentration of cyanide ions. We use AAS with graphite furnace which has sensitivity of 1 ppb for gold. Analyzing samples after QCM we obtained the following results: 0 for 2ppb cyanide in pure water; 1 ppb for 5 ppb cyanide in pure water and 328 ppb for 1 ppm cyanide in pure water. Note that the error is greater for low concentration of cyanide in pure water. Below 50 ppb we get more quality measurement and over 50 ppb we get both quality and quantity measurement. The second procedure is based on reducing the weight of gold electrode by removal gold atoms in solution. Sauerbrey was the first to recognize the potential usefulness of the Quartz Crystal Microbalance (QCM) technology and demonstrate the extremely sensitive nature of these piezoelectric devices towards mass changes at the surface of QCM electrodes.

The results of his work are embodied in the Sauerbrey equation, which relates the mass change per unit area at the QCM electrode surface to the observed change in oscillation frequency of the crystal. Thus, in theory, the QCM mass sensor does not require calibration. However, it must be kept in mind, that the Sauerbrey equation is only strictly applicable to uniform, rigid, thin-film deposits. For this

purpose are measurements of the oscillation frequency sensor before and after testing solution.

These measurements are made in air temperature and humidity controlled. At a temperature control of  $\pm 1^{\circ}\text{C}$  and humidity control of 1% we obtain a sensitivity of 7 ng for mass control, or 0.4 Hz for frequency. If all cyanide ions in solution 1 mL out gold atoms, minimum sensibility will be around 14 ppb concentration of cyanide in pure water. At the first procedure, below 50 ppb we get more quality measurement and over 50 ppb we get both quality and quantity measurement.

### Conclusions for cyanide

This paper provides a method and system capable to measure in real-time the traces of free cyanide in water or in other solutions using a QCM system. The analytical apparatus is simple to operate and does not require special analyst skills. Moreover, this system has the ability to be incorporated into an automated, inline, real-time monitoring system for both immediate response and continuous monitoring. System is based on selective reactivity of cyanide anions with the gold electrode of quartz sensor. The detection limit for static condition at pH 12 and 1mL solution is 5 ppb for analysis times of 6 minute and the detection limit for dynamic condition at pH 12 and flow 0.5 mL/min is 5 ppb for analysis time of 8 minute. The incorporation of the flow cell improves both analyte sensitivity and instrument precision, with an average signal intensity drift of only 5% over a 1h analysis. The calibrations show excellent linearity over a variety of cyanide concentrations ranging from low ppb to hundreds of ppm.

### 4.4. Analysis of copolymer MA-St

New maleic anhydride (MA) copolymer with styrene (St) modified by condensative coupling reaction with azoic photo chrome dye ( $\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{N}=\text{N}-\text{C}_6\text{H}_4-\text{CH}_3$ ) was obtained and the results concerning structures were published in some articles [39].

The selection of the maleic anhydride copolymer is justify by MA tendency for forming alternated copolymers, what permits to obtain architectures with big degree of structural regularity as well as of the anhydridic group reactivity in reaction with primary amines. Chemical transformation of copolymer maleic anhydride-styrene (MA-St) has been realized by two stages: preparation of amic acid and the second, imidization of amic acid. The amic derivative has been obtained by reaction between copolymer and photochrome azoic dye ( $\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{N}=\text{N}-\text{C}_6\text{H}_4-\text{Cl}$ ), molar ratio 1:1, in DMF (0.5 mole/L), 2 hours at room temperature.

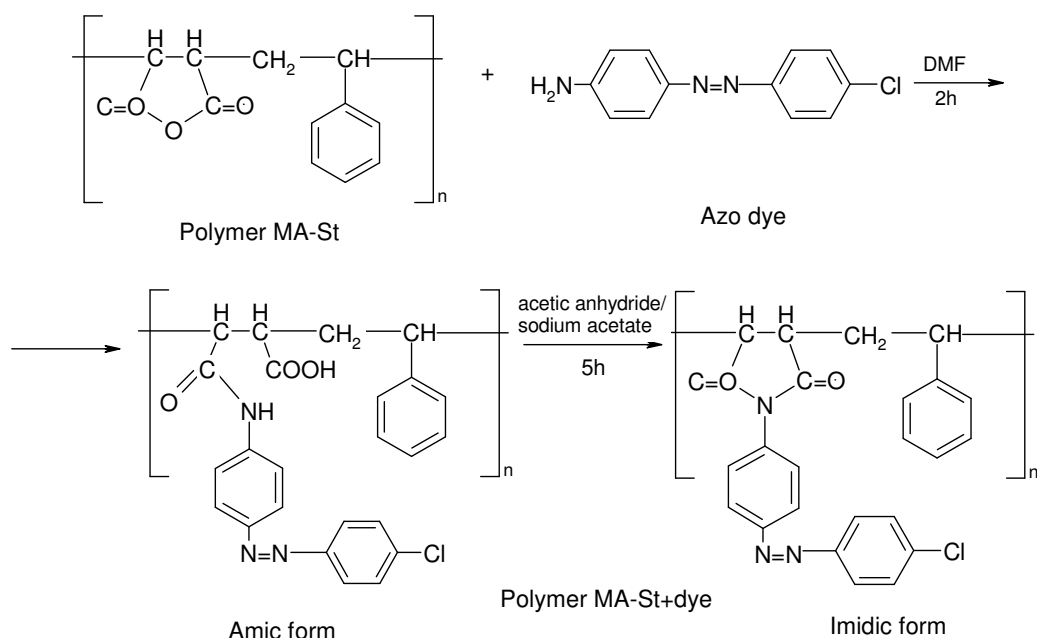
The copolymer (alternated structure with molecular mass obtained by Gel Permeation Chromatography/Light Scattering - Waters 510: tetrahydrofuran,

$M=95000$ , density  $1.174 \text{ g/cm}^3$  and  $T_g = 158^\circ\text{C}$ ) has been prepared by DMF distillation at low pressure and then the polymeric compound has dissolved in acetone.

After filtration of the prepared polymeric solution, under stirring, the precipitation in ethylic ether has been realized.

Consecutively, the imidic form has been obtained by dehydration with acetic anhydride/sodium acetate, at  $60^\circ\text{C}$ , 5 hours, under stirring, as well (figure 25). The yield of the synthesis was  $\sim 40\%$ .

All synthesized compounds are purified and analyzed by spectral analyses (TLC, UV-VIS, IR and NMR) [40].



**Fig. 25.** Structures of polymer MA-St, dye and copolymer MA-St+dye.

Using the condensate coupling reaction can be possible to increase the stability of *sin* isomer by modification of polymeric structure (amic $\rightarrow$ imidic).

In this case it is possible to change the electrical nature of immediate vicinity of photochromic group correlated with amic form of copolymer MA-St changed with photochrome substituted with chlor.

Thermogravimetric analysis (TG) and differential thermal analysis (DTA) demonstrate that the stability of copolymer MA-St+dye, amic form is high comparative with imidic form [41].

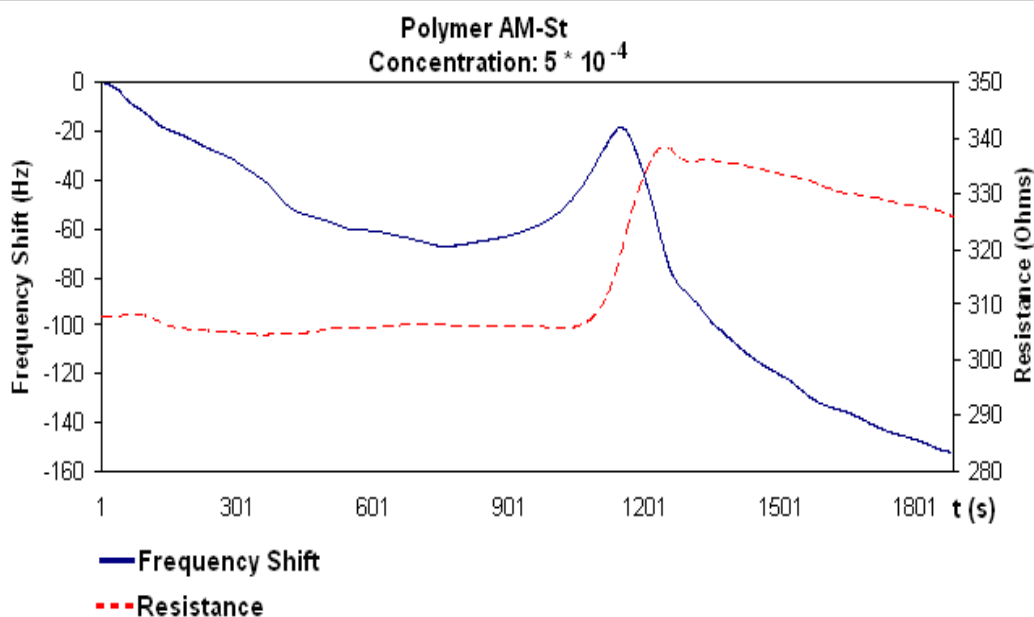
The selection of the maleic anhydride copolymer is justify by MA tendency for forming alternant copolymers, what permits to obtain architectures with big degree of structural regularity as well as of the anhydridic group reactivity in reaction with primary amines.

Quartz crystal microbalance is used to monitor in real-time the polymer adsorption followed by azoic dye adsorption and then copolymer adsorption as well as optimization of interaction processes and determination of solution effects on the analytical signal [42].

Also, the response of the sensor at MA-St copolymer is fast, large, and reversible.

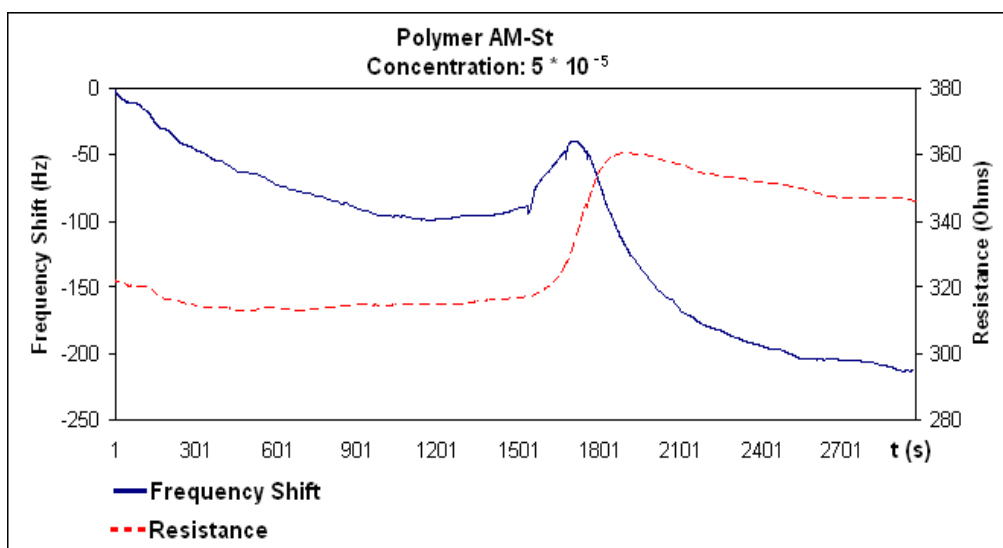
In the figure 26, 27 and 28 we represent the analytical data from Polymer MA-St with QCM static, 1 mL fluid deposition on the gold electrode (CrAu).

Solutions of maleic anhydride (MA) copolymer with styrene (St)  $5 \cdot 10^{-4}$  concentration in Dimethylformide (DMF) are adsorbed at gold electrodes of QCM and the sensor responses are estimated through decrease of QCM frequency after about 1100 seconds (fig. 26).

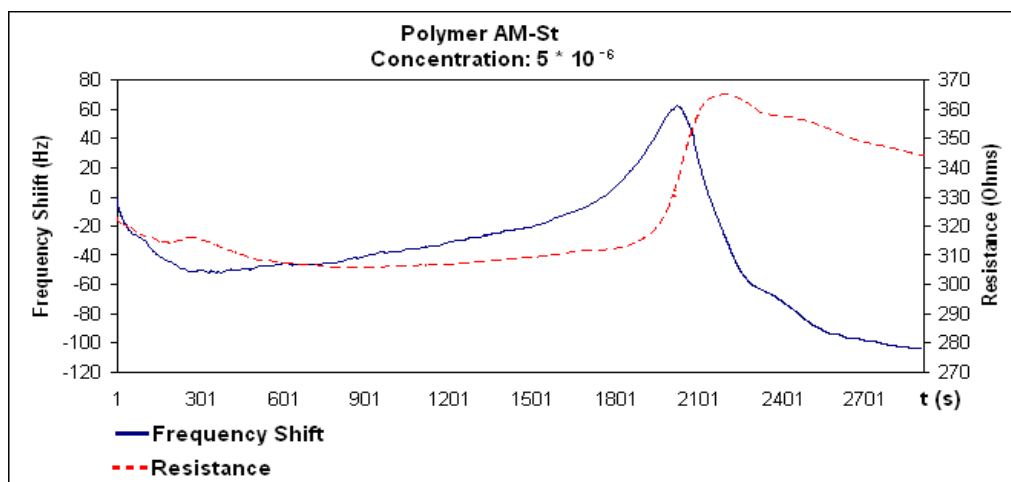


**Fig. 26.** Frequency Shift and resistance versus time with QCM static in Polymer MA-St in DMF ( $5 \cdot 10^{-4}$  concentration).

For maleic anhydride (MA) copolymer with styrene (St)  $5 \cdot 10^{-5}$  concentration (figure 27) decrease of QCM frequency is after 1650 seconds and for maleic anhydride (MA) copolymer with styrene (St)  $5 \cdot 10^{-6}$  concentration (figure 28) decrease is after 1950 seconds.



**Fig. 27.** Frequency Shift and resistance versus time with QCM static in Polymer MA-St in DMF ( $5 \cdot 10^{-5}$  concentration).



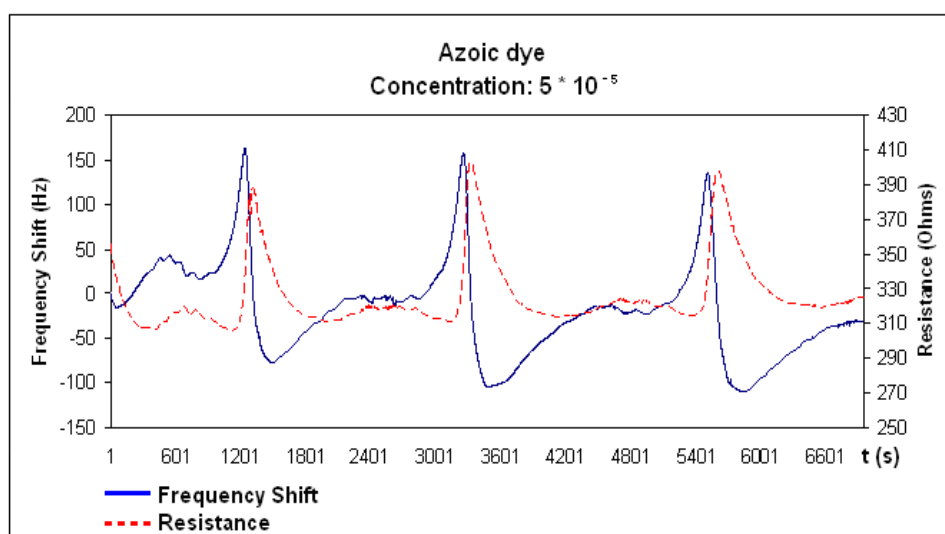
**Fig. 28.** Frequency Shift and resistance versus time with QCM static in Polymer MA-St in DMF ( $5 \cdot 10^{-6}$  concentration).

Solutions of azoic dye  $5 \cdot 10^{-4}$  concentration in Dimethylformide (DMF) are adsorbed at gold electrodes of QCM and the sensor responses are estimated through decrease of QCM frequency (figure 27).

In this figure we can see that the adsorption on gold electrode of QCM is followed by desorption after one period of time, about 2400 seconds.

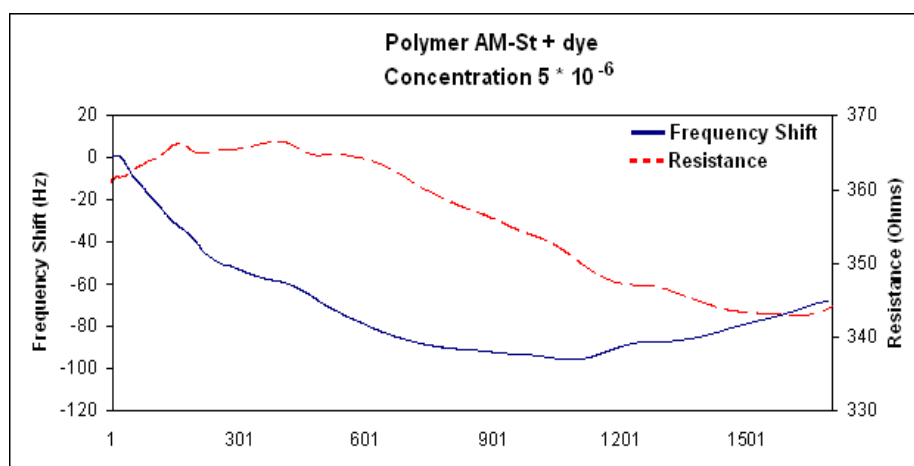
On the gold electrode we have a dynamic and reversible process and. This process depends by concentration of azoic dye and type of diluents.





**Fig. 29.** Frequency Shift and resistance versus time with QCM static in Azoic dye in DMF ( $5 \times 10^{-5}$  concentration).

If we mix the Copolymer MA-St with Azoic Dye we obtain the result that is showed in the figure 30. In this case we have a very stable compound polymer. Using the condensative coupling reaction can be possible to increase the stability of *sin* isomer by modification of polymeric structure (amic  $\rightarrow$  imidic). In this case it is possible to change the electrical nature of immediate vicinity of photochromic group correlated with amic form of copolymer MA-St changed with photochrome substituted with chlorine. Thermogravimetric analysis (TG) and differential thermal analysis (DTA) demonstrate that the stability of copolymer MA-St+dye, amic form is high comparative with imidic form.



**Fig. 30.** Frequency Shift and resistance versus time with QCM static in Polymer AM St mixed with Azoic dye in DMF ( $5 \times 10^{-5}$  concentration).

By using QCM technique the important physical and chemical properties of dye and polymer can be followed by observing the associated mass changes.

The conformational modification at azoic sequence level (anti sin anti) for dye and polymer represent the fundament of information registration and conservation.

The sensor response is influenced by viscoelastic properties of the adhered organic compounds, especially copolymer.

The stability of *amic*→*imic* structure and conformational modification of copolymer, which are demonstrated by thermogravimetric and spectral analyses (UV-VIS, IR, NMR), is alized by using QCM technique used especially to monitor the adsorption of organic the Cr-Au sensor with small modification of usually parameters (resistance and time).

The IR and NMR spectra are demonstrated the viability of the structures proposed for olymer and copolymer (M=96000).

In IR spectra the absorption bands proper to the vibration pecific groups for each synthesized compound were identified (Figure 31).

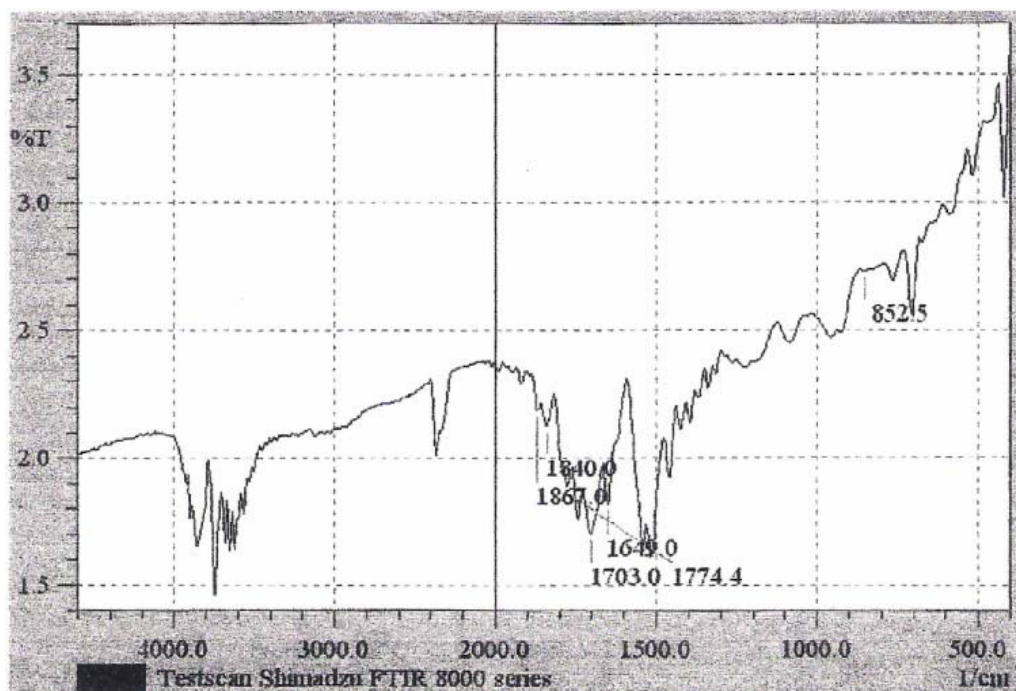


Fig. 31. IR spectra.

The  $^{13}\text{C}$ —NMR spectra content the information about carbon atoms; the values of ery interesting information about carbon atoms from carboxyl group and carbonyl group (figure 32).

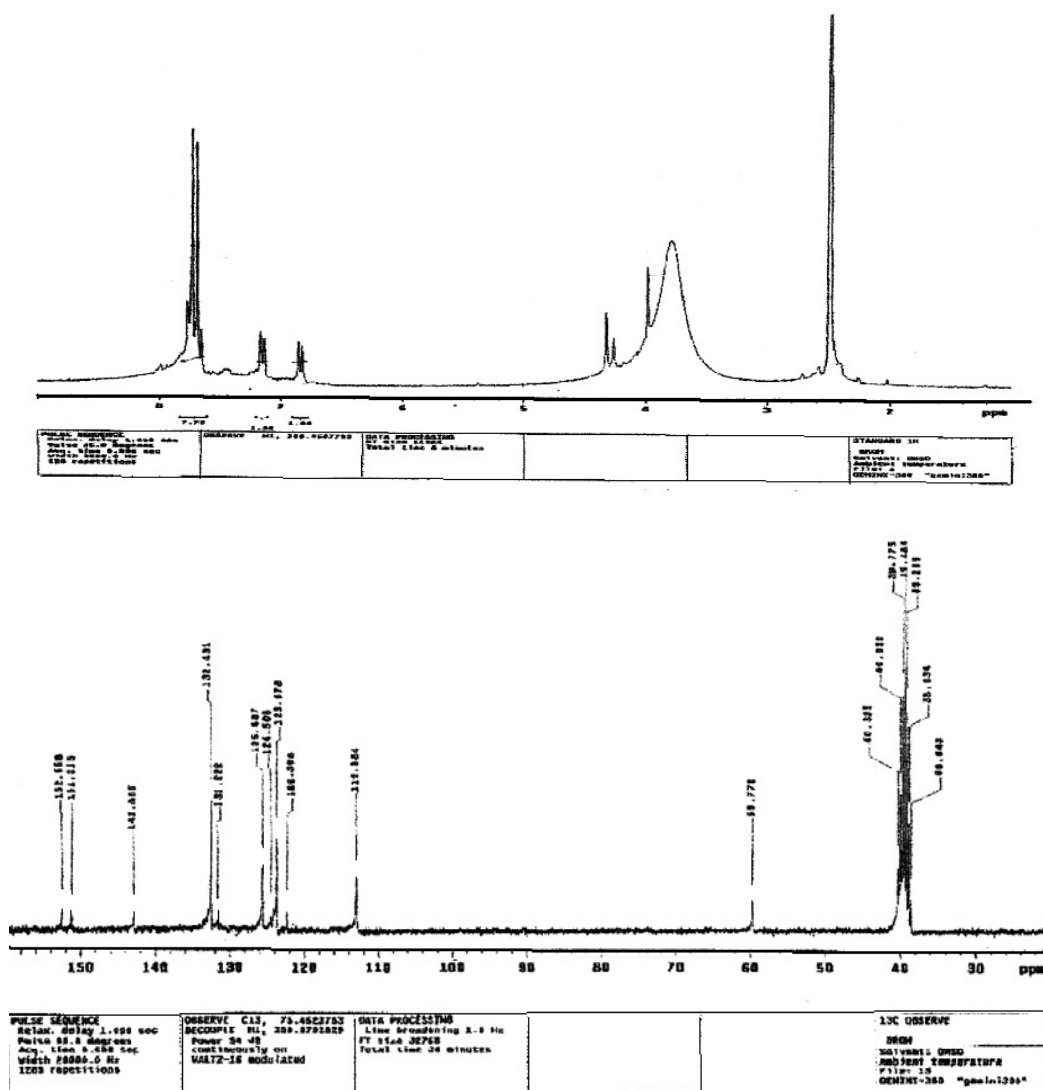


Fig. 32. NMR spectra for copolymer MA+St+dye: (a) 1H NMR; (b) 13C NMR.

By using QCM technique the important physical and chemical properties of dye and polymer can be followed by observing the associated mass changes. The conformational modification at azoic sequence level (anti sin anti) for dye and polymer represent the fundament of information registration and conservation. The sensor response is influenced by viscoelastic properties of the adhered organic compounds, especially copolymer. The stability of *amic*→*imic* structure and conformational modification of copolymer, which are demonstrated by thermogravimetric and spectral analyses (UV-VIS, IR, NMR), is alized by using QCM technique used especially to monitor the adsorption of organic the Cr-Au sensor with small modification of usually parameters.

#### 4.5. Self-assembled monolayer by quartz crystal microbalance

Self-assembling process of alkylthiols on gold is initiated by the strong chemical interactions between the sulfur and gold surface. This interaction is considered a result of chemisorption that forced a thiorate molecule to adsorb commensurate with a gold lattice. Then, the tail-to-tail interaction of the molecules created by lateral interchange no bonded interactions, such as by van der Waals, steric, repulsive and electrostatic forces, is strong enough to align the molecules parallel on the gold surface and create a crystalline film.

In most cases, organic disulfides, thiols and sulfides have been utilized for the preparation of stable SAMs on the gold surfaces. Self-assembled monolayer (SAM) techniques offer a new strategy for the immobilization of antibodies, antigens and DNA on the surface of quartz crystal electrodes.

The Quartz Crystal Microbalance (QCM) system provides a unique opportunity to monitor, in-situ and in real-time, the rate of adsorption of ethanthiol onto gold. The change in frequency resulting from the adsorption of ethanthiol molecules onto the gold electrode of a QCM crystal allows direct measurement of the rate of formation of Self Assembled Monolayer (SAMs) [43-47].

The IR Spectroscopy with attenuated total reflectance (ATR) confirms the formation of the Au-thiolate bonds in the CH stretching region. A self-assembled monolayer (SAM) is defined as a two-dimensional film, one molecule thick, covalently assembled at an interface. The classical example of a SAM is the reaction of alkane thiols with a gold surface. Since sulphur donor atoms strongly coordinate on metal substrates (Au, Ag, Pt, etc), sulphur containing molecules such as disulfides (R-SS-R), sulphides (R-S-R) and thiols (RSH) can form various functionalized SAMs with highly organized and compact construction.

For example, when a clean gold surface is immersed in an alkane thiol solution, the following self-assembly chemisorption reaction takes place:



This reaction self terminates at one monolayer, when all accessible gold sites are consumed. The Langmuir adsorption model has been applied successfully to model the adsorption/desorption kinetics of these processes. The self-assembly of alkanethiol monolayer onto gold has been studied extensively due to the potential application of these interfaces to (1) chemical and biological sensing, (2) nano- and micro-device manufacturing and (3) surface modification. A significant amount of work has been dedicated to understanding the steady-state properties of SAMs once they have formed. Alkane thiol [ $\text{CH}_3 (\text{CH}_2)_{n-1}\text{SH}$ ] molecules are spontaneously arranged on an Au substrate and formed into self-assembled monolayers (SAMs).

In our experience we use 1% ethanthiol ( $\text{HS-CH}_2\text{-CH}_3$ ) in ethanol ( $\text{CH}_2\text{-CH}_3\text{-OH}$ ). We put on quartz crystal sensor this solution and turn on equipment for oscillating frequency.

Thermodynamics of Self-Assembled Monolayer on a Substrate is based on its free energy. The free energy is minimized because of three main processes [1]:

- Chemisorption of the surfactant onto the surface followed by organization (S), about 40-45 Kcal/mol;
- Inter-chain van der Waals interaction and electrostatic interaction ( $\text{CH}_2$ ), < 10 Kcal/mol;
- Terminal Functionality ( $\text{CH}_3$ ), about 0.7-1.0 Kcal/mol.

In the figure 33 we show the spontaneous organization of molecules on substrate.

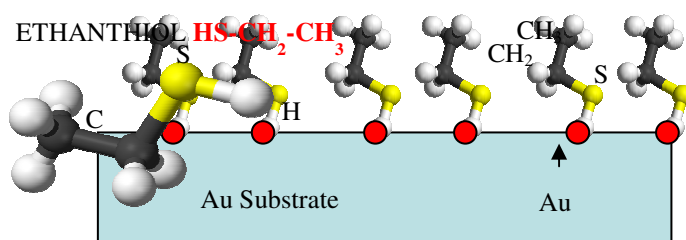


Fig. 33. Self-Organization of ethanthiol on the Au substrate.

In the figure 34 we show the crystalline structure of the gold of sensor by Wide Angle X-Ray Diffraction (WAXRD) before processing SAM. The distance between gold atoms for (111) orientation is 2.36 Å and for (222) orientation is 1.18 Å, so that the number of atoms on surface is about  $10^{16}/\text{cm}^2$ .

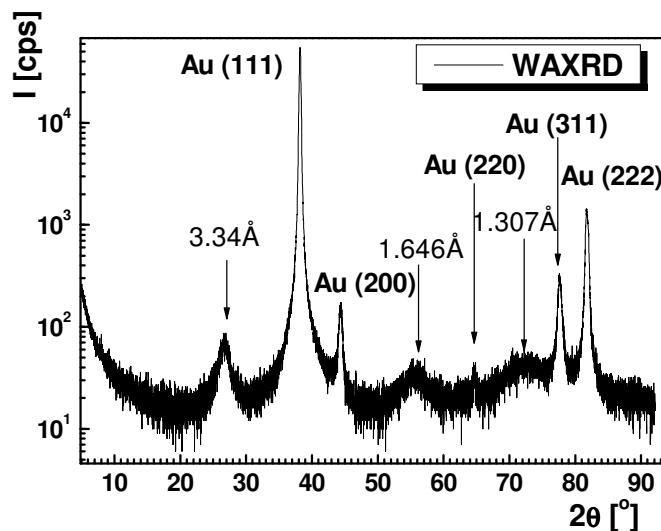
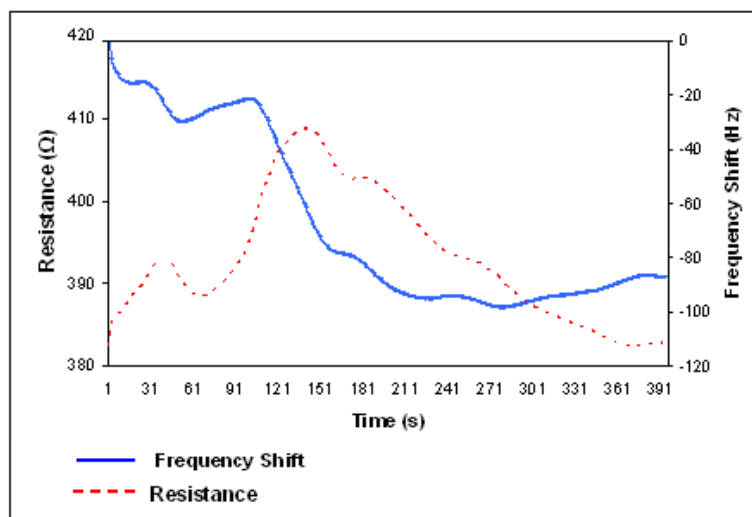


Fig. 34. WAXRD on CrAu quartz sensor.

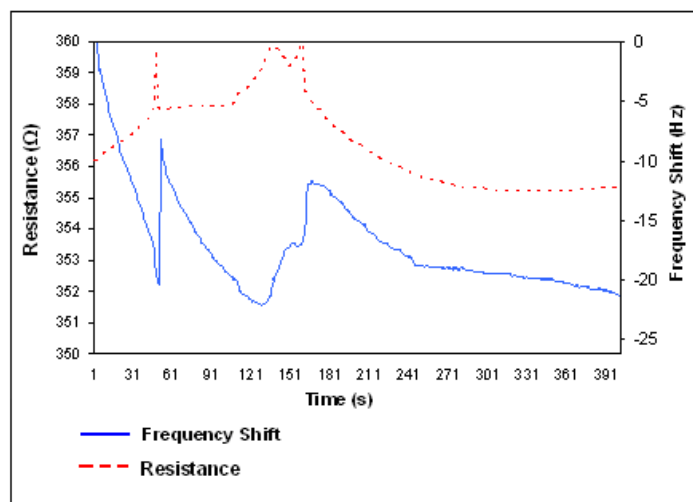
## 5. Results and discussions

We use QCM and FTIR Spectroscopy analysis for the novel application. The standard QCM System (QCM 200 from Stanford Research Systems) is a stand-alone instrument with a built-in frequency counter, resistance meter, personal computer and printer. It includes controller, crystal oscillator electronics, crystal holder, and quartz crystals. In addition, the QCM has an RS-232 interface and comes with both Windows and Lab-VIEW software providing real-time display, analysis and storage of our QCM data. The results after monitoring in real-time we obtained the following characteristics expressed in the figure 35 and 36. For CrAu sensor (figure 35), self-assembled monolayer open after 90 seconds and finish after 270 seconds.



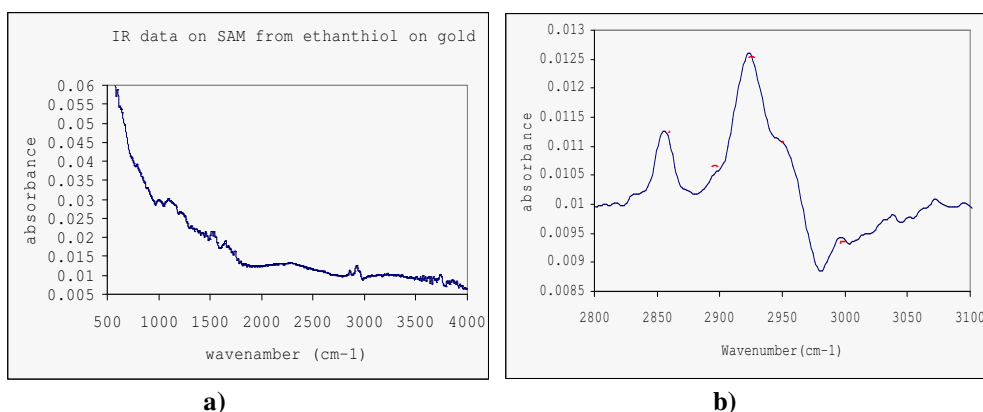
**Fig. 35.** The QCM diagram “Frequency shift versus time” and “Resistance versus time” for 1%  $C_2H_5SH$  in  $C_2H_5OH$  on CrAu substrate

In this time all gold atoms from surface (about  $10^{16}$  atoms) couple with  $S-CH_2-CH_3$  radicals. Supplementary mass attached on surface is give by -60Hz frequency shift, or 1080ng ( $60 \times 18ng/Hz$ ), or  $180 \times 10^{-10}$  mol, in concordance with the theory. For TiAu sensor (figure 36), self-assembled monolayer open after 61 seconds and finish after 241 seconds. In this time all gold atoms from surface (about  $10^{16}$  atoms) couple with  $S-CH_2-CH_3$  radicals. Supplementary mass attached on surface is give by -20Hz frequency shift, or 320 ng ( $20 \times 18ng/Hz$ ), or  $55 \times 10^{-10}$  mol. In this case we obtained less than CrAu. An explication is that the crystalline structure of the gold on Ti not permits more bonds with  $S-CH_2-CH_3$  radicals than the crystalline structure of the gold on Cr. In the both cases we use 1 ml solution of 1% ethanthiol ( $HS-CH_2-CH_3$ ) in ethanol ( $CH_2-CH_3-OH$ ), about  $1.3 \times 10^{-4}$  mol of ethanthiol, enough for forming monolayer.



**Fig. 36.** The QCM diagram “Frequency shift versus time” and “Resistance versus time” for 1%  $C_2H_5SH$  in  $C_2H_5OH$  on TiAu substrate.

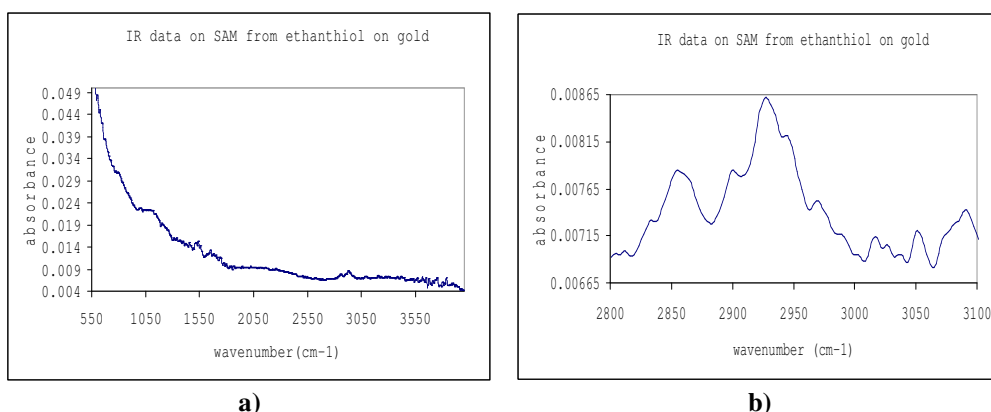
With QCM Self-assembled is successful because we put a right balance between *attractive forces* (that tend to stick the components together) and *Brownian motion* (thermal agitation that tend to pull them apart). A large number of structures are assembled at a time, and we can control of many of them are made by how many molecules we put in the flask. The components are continuously coming into place and breaking apart. For FTIR Spectroscopy we use Tensor 27, from Bruker with ZnSe attenuated total reflectance (ATR). The IR radiation from the spectrometer enters the ZnSe crystal and then reflects through the crystal and penetrating “into” the sample a finite amount with each reflection along the top surface via the so-called “evanescent” wave. The typical spectrums are shown in the figure 37 and 38, where we represent the IR data on ethanthiol on gold for two sensors: CrAu/quartz and TiAu/quartz.



**Fig. 37.** IR data of SAM from ethanthion on gold (CrAu/quartz Substrate):  
a) all spectrum and b) spectrum between  $2800\text{ cm}^{-1}$  and  $3100\text{ cm}^{-1}$ .

The beam is directed out of the crystal and back into the normal beam path of the spectrometer. In this case the transmittance is identical with reflectance. The method is the best for our monolayer of ethanthiol on gold. The IR spectral interval is between  $650\text{ cm}^{-1}$  and  $4000\text{ cm}^{-1}$ . For each FTIR spectrum, data was collected using 1700 scans with  $2\text{ cm}^{-1}$  resolution.

The CH stretching vibrations of the alkyl chain are very sensitive to packing density and to the presence of Gauche defects, which makes them ideally suited as probes to determine SAM quality. In particular, we obtaining achieve an antisymmetric  $\text{CH}_2$  stretching vibration (d-) at  $2925\text{ cm}^{-1}$  which is useful indicator.



**Fig. 38.** a) IR data of SAM from ethanthion on gold (TiAu/quartz Substrate): all spectrums.  
b) IR data of SAM from ethanthion on gold (TiAu/quartz Substrate):  
spectrum between  $2800\text{ cm}^{-1}$  and  $3100\text{ cm}^{-1}$

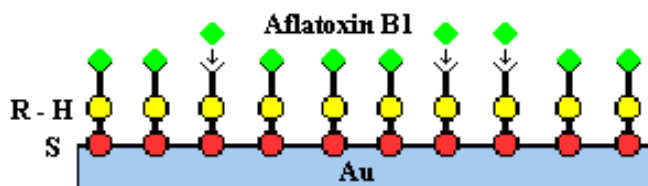
The absence of S-H stretching mode ( $2580\text{ cm}^{-1}$ ) in the IR spectrum, confirms that the Au-S chemical bond is formed. Thus, the spectral characteristics of the IR spectrum demonstrate that the ethanthiol molecules are absorbed into the gold nanoparticles through sulphur atoms.

#### 4.6. Detection of aflatoxin in food

SAM of alkythiols on gold use for many applications, one of this is in detection of aflatoxin from food [48, 49]. Quartz Crystal Microbalance is an ultrasensitive piezoelectric method for detection of aflatoxin in concentrations under the value  $4\text{ ng/ml}$  (ppb) – current maximum permitted levels set by European Commission. QCM crystals are used as sensors to determine mass changes as a result of frequency changes and becoming into a good alternative analytical method in a great deal of applications such as biosensors, analysis of biomolecular interactions, study of bacterial adhesion at specific interfaces, pathogen and microorganism detection, study of polymer film-biomolecule or cell-substrate interactions, immunosensors and extensive use in fluids and polymer characterization and electrochemical applications among others.

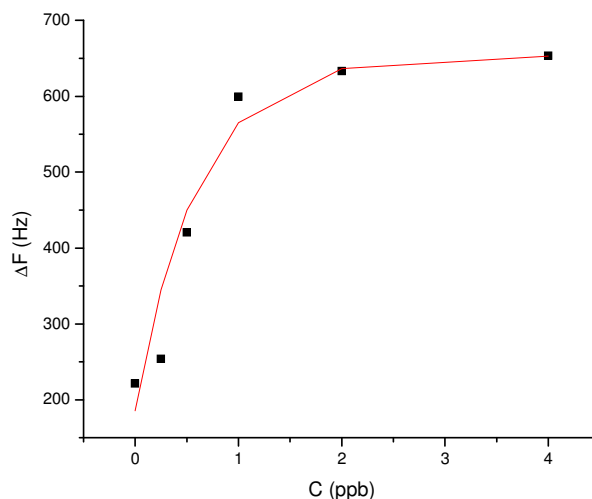


The sensor can be covered with a monolayer by self-assembled monolayer (SAM) to increase the sensibility and to detect some bio- macromolecules. SAM was obtained from ethanethiol (concentration 1%) by QCM, on a TiAu electrode [50]. In figure 39 is presented the attachment mode of aflatoxin B1 on SAM with alkythiols.



**Fig. 39.** Quartz sensor with SAM used for detection of aflatoxin B1.

Our samples was obtained from standard solution 4 ppb (Charm Science) diluted in methanol (ICCF Bucharest). We obtained a calibration curve for aflatoxin B1 with a TiAu electrode covered with SAM. In the figure 40 is presented the dependence between frequency shift and aflatoxin B1 concentration.



**Fig. 40.** Frequency shift vs. aflatoxin B1 concentration.

After plotting with Origin 7 software, the correlation equation of frequency shift with concentration was obtained:

$$\Delta F = 653.3 - 467.558 \cdot e^{-C/0.59976}$$

In the future, the calibration curve will help us to determine the aflatoxins concentration in any type of milk (human milk, powder milk or fresh milk).

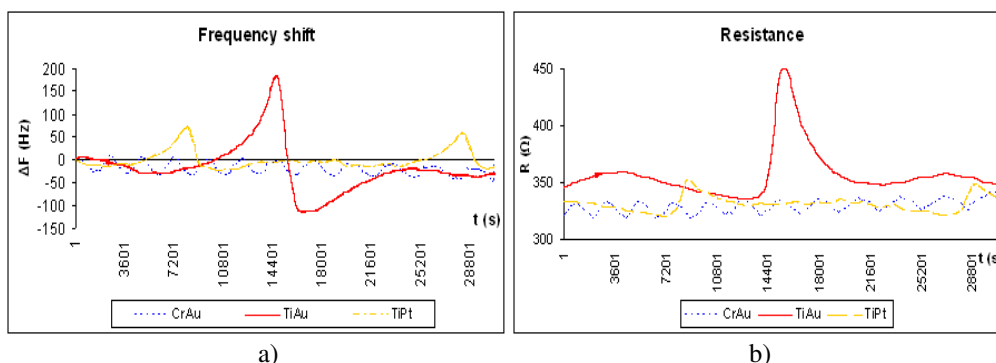
#### 4.7. QCM for characterization Mushrooms (*Armillaria Mellea*)

*Armillaria Mellea* is the most common mushrooms in our country. Grow on tree, their root, by them, because it produces parasites and white mold. We find in coniferous forests, deciduous orchards since August.

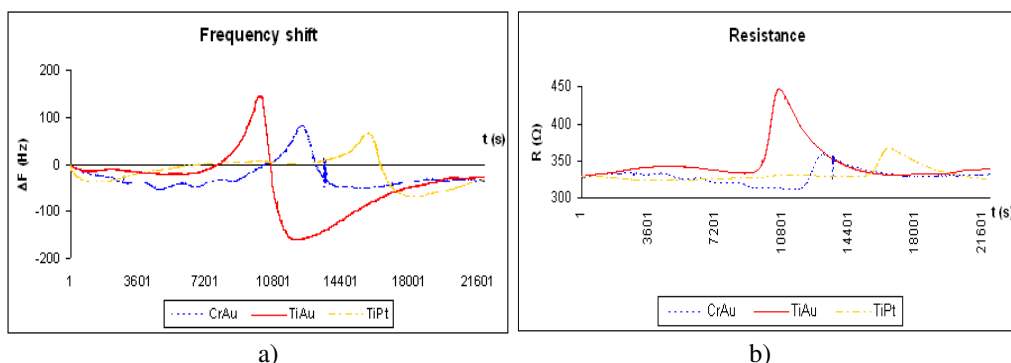
They can be used both for human food and as bio-indicators of environmental air pollution to determine toxic heavy metals. In general, there is no analytical method for mushrooms that we indicate their degree of toxicity. Using QCM we can to determine the degree of toxicity obtained by interpreting graphs. Any modification of the contents of the fungus is observed by varying the resonant frequency and resistivity liquid containing material prepared. Measuring solutions samples are obtained by microwave digestion fungi and by dilution with pure water to concentrations between 1 and 500 ppm. Using QCM for characterization mushrooms, we quickly understanding the quality to be edible and environmental in which have been developed. For the experiments we used several types of QCM sensors: CrAu, TiAu and TiPt. The results are presented in the figure 41 and 42.

Of the two charts the following conclusions:

- 1) sensor sensitivity depends of metallization electrodes. The sensors with TiPt and TiAu have the highest sensitivities. It follows that titanium has the highest adsorption for mushrooms;
- 2) response time is shorter as the concentration is lower.



**Fig. 41.** The QCM diagram a)Frequency shift versus time and b)Resistance versus time for mushrooms (Armillaria Mellea) with  $5 \cdot 10^{-4}$  concentration (500 ppm).



**Fig. 42.** The QCM diagram a)Frequency shift versus time and b)Resistance versus time for mushrooms (Armillaria Mellea) with  $5 \cdot 10^{-6}$  concentration (5 ppm).

As a general conclusion, we can say that: QCM sensor can be used in the characterization of fungi.

As the solution is diluted with, so we get the result faster and with great sensitivity. Know that it is very important balance calibration with known standard solutions.

In environmental analysis mushrooms are good bio-indicators because the mushrooms determine air contamination with heavy metals.

Mushrooms as part of the heavy metals accumulate in the growth from the surrounding air. The QCM analyzes are qualitative analysis.

The name and the quantity of heavy metals are made by other methods of spectrographic analysis (AAS, ICP-MS, WDXRF).

#### 4.8. Biodegradable polymers

Biodegradable polymers contain polymer chains that are hydrolytically or enzymatically cleaved, resulting in soluble degradation products [51]. Even though biodegradable polymers have numerous applications, there are properties that tend to be common among them. All biodegradable polymers should be stable and durable enough for use in their particular application, but upon disposal they should easily [break down](#).

Other properties of biodegradable polymers that are common among those used for medicinal usages include: non-toxic; capable of maintaining good mechanical integrity until degraded, and capable of controlled rates of degradation.

One of the most important and most studied groups of biodegradable polymers is [polyesters](#) [52].

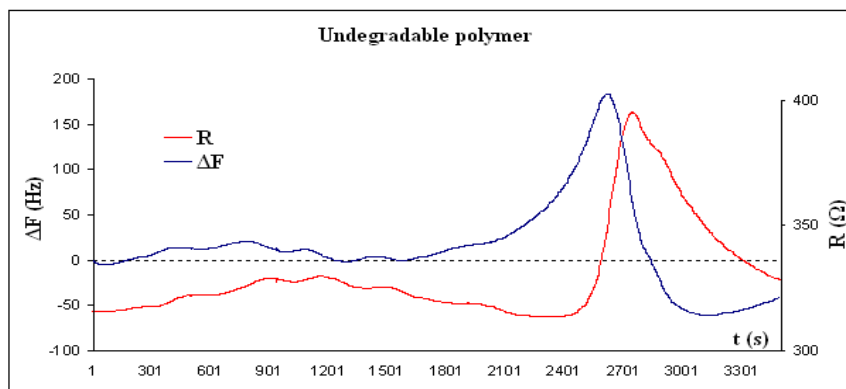
In general, biodegradable polymers break down to form gases, salts, and [biomass](#). Complete [biodegradation](#) is said to occur when there are no [oligomers](#) or [monomers](#) left.

The breakdown of these polymers depends on a variety of factors including the polymer and also, the environment the polymer is in.

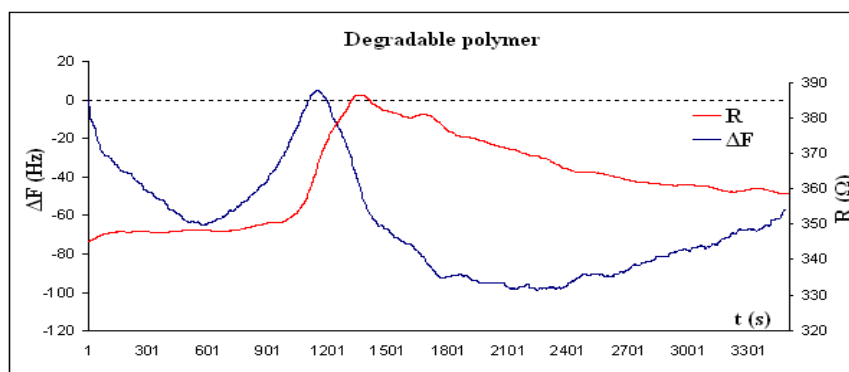
Polymer properties that influence degradation are: [bond](#) type, [solubility](#), and [copolymers](#) among others. The surrounding environment of the polymer is just as important as the polymer structure itself. These factors included items such as the [pH](#), [temperature](#), [microorganisms](#) present, and [water](#) as just a few examples.

In the paper we present the response shift frequency and resistance with QCM for three types of polymers: non-degradable polymers, degradable polymers obtained by syntheses and natural biopolymers.

All samples were obtained by microwave digestion and  $10^{-6}$  concentration (1 ppm) mixed with pure water. Results are presented in the figure 43, 44 and 45.



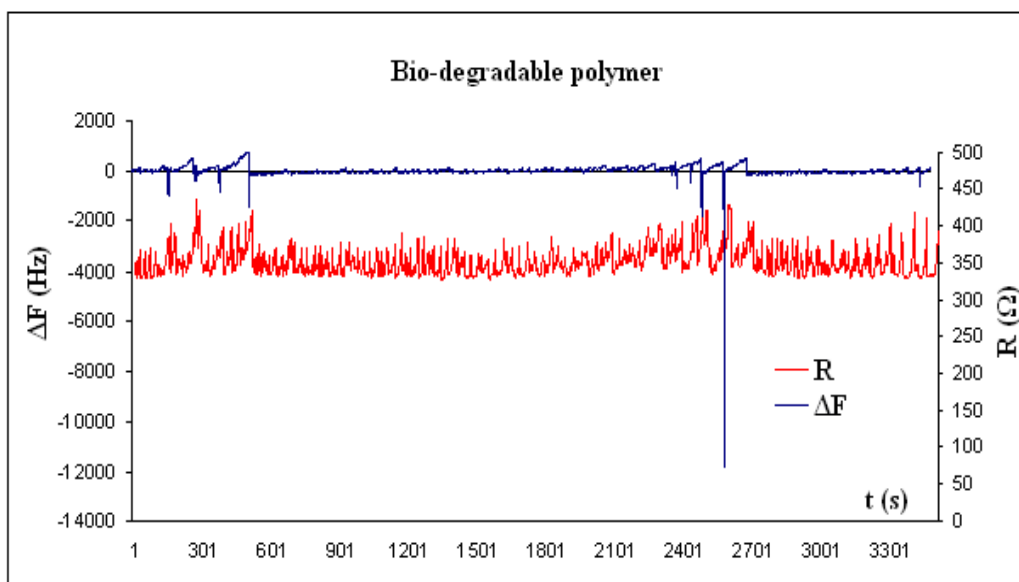
**Fig. 43.** The QCM diagram "Frequency shift versus time" and "Resistance versus time" for undegradable polymer.



**Fig. 44.** The QCM diagram "Frequency shift versus time" and "Resistance versus time" for degradable polymer.

In Figures 43 and 44 shows that the maximum time for the liquid adsorption degradable polymer is approximately two times faster than liquid polymer undegradable. As degradable material content is higher the more time shortens (2500 s versus 1200 s). All of these graphs is clearly seen polymers undegradable, that a rejection of the molecules of the polymer (frequency increases while the mass decreases comparing the oscillation frequency at time zero), while the polymers degradable occurs adsorption (frequency decreases while mass increase comparing the oscillation frequency at time zero). The explanation is quite simple: there is degradable polymer decomposition while under the influence of vibration energy of QCM. Biopolymers biodegradable represent a field current scientific research of great importance to ecological, scientific and economic. But continuous increase concern for the environmental consequences of the products of such materials when they end up in landfills after fulfilled their role. Especially problematic materials, disposables such as packaging. Macromolecules with high molecular weight, contain covalent bonds are not easily decomposed naturally, in conditions offered by infrastructure waste management.

Polymeric materials produced from natural biopolymers, such as polysaccharides (such as starch and cellulose) protein, triglycerides (vegetable oils), generally agricultural products are biodegradable and can play a significant role in solving environmental problems raised by the use of polymeric materials. Biodegradable polymers can be obtained by biosynthesis of bacterial natural materials (polyester polysaccharide), or by chemical synthesis of natural materials renewable (polyesters from lactic acid - obtained by fermentation from starch. The products Mixed based on synthetic polymers and biopolymers can also present a greater or lesser biodegradability. Figure 45 is obtained by QCM represents diagrams for a biodegradable material obtained by synthesis based on cornstarch. Concentration of the solution is also  $10^{-6}$  (1 ppm).



**Fig. 45.** The QCM diagram “Frequency shift versus time” and “Resistance versus time” for biodegradable polymer.

From figure 45 is clearly seen rapid change in frequency and resistivity of the solution. There is a process of attraction and rejection molecules as biodegradable material such as a move as a game of Ping-Pong. Degradation is very fast in the aqueous medium and high frequency vibration (5 MHz). Energy device of QCM accelerates degradation of biopolymer.

## 5. Applications with QCM

**Biotechnology:** a) Interactions of DNA and RNA with complementary strands; b) Specific recognition of protein ligands by immobilized receptors, immunological reactions; c) Detection of virus capsids, bacteria, mammalian cells; d) Adhesion of cells, liposomes and proteins; e) Biocompatibility of surfaces; f) Formation and prevention of formation of biofilms.

**Drug Research:** 1) Dissolution of polymer coatings; 2) Molecular interaction of drugs; 3) Cell response to pharmacological substances; 4) Drug delivery.

**Other applications:** 1) Immunosensors; Sorption sensors; Moisture analyzers; 2) Particulate monitors; 3) Contamination monitors; 4) Electrovalency measurements; 5) Hydrogen absorption on metal films; 6) Bubble formation; 7) Redox and conductive polymer research; 8) Double-layer characterization; 9) Corrosion studies; 10) Surface oxidation; 11) DNA and RNA hybridization studies; 12) Antigen-antibody reactions; 13) Protein adsorption; 14) Detection of virus capsids, bacteria, mammalian cells; 15) Biofouling and antifouling; 16) Biomembranes and biomaterials; 17) Protein-protein interactions; 18) Self-assembled monolayers (SAMs); 19) Molecularly imprinted polymers (MIPs); 20) Langmuir/Langmuir-Blodgett films; 21) Laser ablation, desorption and breakdown studies; 22) MEMS nanomaterials; 23) Intelligent biomaterials.

Also, in research environment, the most common QCM crystal applications include metal deposition monitors, chemical reaction monitors, biomedical sensors, and environmental monitoring applications etc. Other applications include detection of mass, density, viscosity, adsorption, desorption, and corrosion [1, 4-6]. Eventually, the QCM was used also in gaseous environments, e.g. as gas and humidity sensors and for the detection of aerosols [53, 54].

## 6. Conclusions

The widespread use of the quartz crystal microbalance (QCM) can be attributed to its excellent sensitivity to the properties of liquid and soft solid materials with which it is brought into contact. This sensitivity arises from the coupling between the mechanical, shear oscillation of the crystal and its electrical response at frequencies close to resonance. This coupling depends on the details of the shear wave propagation into the material with which the QCM is in contact.

For most liquid and soft viscoelastic materials, the decay length of this shear wave is on the order of 100 nm; so in these cases, the QCM is truly a surface-sensitive technique. This surface sensitivity is responsible for a wide range of applications in biology and electrochemistry, where the QCM is often used to sense material that is absorbed to the crystal surface from solution.

The material property that determines the response of the QCM is the complex acoustic impedance of the loading material.

In many cases, the acoustic impedance varies with the distance from the crystal surface, and models accounting for this depth dependence must be utilized in order to interpret the data.

The monitoring the frequency shift and the resistance shift we monitor the density of the liquid in contact with the electrode and the viscosity of the liquid in contact

with the electrode. QCM device applies directly to adsorption phenomena, but can be modified to any slowly equilibrating system. Several cases are evaluated with emphasis on chemical analyze detection. We report and demonstrate a new approach using a QCM to quickly fabricate high-quality thiol-terminated SAMs on gold. Self-assembled structures by QCM are dynamic. In the QCM initial adsorption of ethanthiol on gold is fast (seconds), then an organization phase follows which should be allowed to continue for minimum 5 minute for best results. SAMs have been thoroughly characterized using FTIR spectroscopy. It has been clearly shown that SAMs with an alkane chains form well-ordered and dense monolayer on Au (111) surface.

QCM is also, an electro-acoustic method suitable for mass and viscoelastic analysis of adsorbed protein layers at the solid/water interface. Proteins at the water/QCM surface interface can also be quantified with  $f$  determination, but adsorbed protein layers also have some degree of structural flexibility or viscoelasticity, that are invisible at simple  $f$  determination.

Viscoelasticity can, however, be visualized by measuring the energy loss, or dissipation of the shear movement of the crystal in water. A new principle of measuring dissipation is to drive the crystal with A/C current at the resonant  $f$  followed by disconnection and analysis of the resulting damped sinusoidal curve. This new invention of pulse assisted discrimination of  $f$  and dissipation makes QCM analysis of adsorbed protein layers very simple and gives unique information about the hydrodynamic conductivity of the adsorbed protein layers and surrounding water.

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