**Proceedings of the 2003 IEEE International Frequency Control Symposium and PDA Exhibition Jointly with the 17th European Frequency and Time Forum**

# ABOUT THE AFFINITY INTERACTION OF BIOSENSOR AND PRECISION OF THE DYNAMICAL FREQUENCY RESPONSE MEASUREMENT

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*Abstract* – **The paper deals with the formation of immunocomplex ASC at the surface of the AT-cut quartz resonator vibrating in thickness-shear mode. The biochemical affinity process can be characterized by association $k_a$ and dissociation $k_d$ kinetic rate constants, the binding between a covalently immobilized small molecule and its relative antibodies. The bindings curves of series resonance frequency $f_s$ vs. time $t$ were measured and discussed. The following problems are considered: activation by cystamine, glutaraldehyde, and albumine as bioactive buffer, ASC antibody solution with phosphate buffer, and regeneration of the biosensor. The precise measuring technique using Agilent E5100A network analyzer was used for this experiment. The possibility of identification of micro-balanced state in liquids is the second interesting case.**

## I. INTRODUCTION

It is know that the chemical and biological sensors based on the BAW resonators (namely quartz) are characterized by analytic-selective coatings, and they are reported for liquid states of different density and viscosity [1]. The dynamic frequency response reports about the properties of the biological liquid medium [2].

## II. RESONANCE FREQUENCY OF THE LOADED QUARTZ RESONATOR

The output parameter of the piezoelectric resonator as biosensor is the resonance frequency. For a small electromechanical coupling factor $k_{26}$ (for quartz typically) the resonance frequency of an AT-cut quartz resonator can be expressed by the relation [3]

$$f_n = \frac{n}{4h}\sqrt{\frac{c_{66}^D}{\rho}}\cdot\left(1-\frac{4k_{26}^2}{n^2\pi^2}-R\right), \quad (1)$$

where $n$ is the overtone order, $h$ is the thickness of the plate, $\rho$ is the density of the unstressed plate, $c_{66}^D$ is the elastic stiffness by constant electric displacement, and $k_{26}$ is electromechanical coupling coefficient

$$k_{26}^2 = \frac{e_{26}^2}{c_{66}^D \varepsilon_{22}^S}. \quad (2)$$

In Eq. (1) $R$ is the mass-loading of the plate caused by the deposited electrodes

$$R = \frac{2\rho' h'}{\rho h}, \quad (3)$$

where $\rho'$ is the density of electrodes and $h'$ is the thickness of electrodes in the symmetrically case. The changes in frequency due to mass-loading of the surface of electrode are mostly expressed by Eq. (4) too.

$$\Delta f = -\frac{2f_r^2 \Delta m}{A\sqrt{\rho_q \cdot \mu_q}}, \quad (4)$$

where $m$ is mass, $A$ area of electrode, $\rho_q$ quartz density, and $\mu_q$ elastic coefficient.

## III. ROTATED Y-CUT QUARTZ RESONATORS AS THE TRANSDUCERS FOR BIOSENSING

The thickness-shear mode of vibrations of rotated Y-cut quartz resonator is mostly used. The AT-cut quartz plano-parallel resonator with gold electrodes on bottom and top surfaces was used in our studies. It was shown that after absorption of substances on the top surface of electrode, a shift $\Delta f$ of the resonance frequency $f_h$ occurs which is directly proportional to the change of loading mass $\Delta m$. It is know that in solution the resonance frequency is also affected by the viscoelastic properties of the surrounding environment. When measuring in the complex samples, the viscosity of the carrier buffer should be near to the viscosity expected for the sample. In order to measure the necessary parameters of the one side loaded resonator, the samples and measured set was

prepared. To improve the reliability of experiments, it is desired to measure the quality factor $Q$ to characterize viscosity effects, or the amplitude $|Z|$ and phase φ of the crystal impedance.

The two classical method of measurement of resonance frequency $f_h$ are used: active method which lead to determinate only one parameter, if a simple measuring set is used.

Passive methods are based on measuring the transmission function of the crystal unit in the defined measuring circuit (π-network). It is know, that the amplitude of the output signal does not exactly the resonance frequency as the measuring of the impedance phase, which gives the value of the resonance frequency up one order more exactly. From this reasons, the recent network analyzers use passive method determining exactly the resonance frequency from the phase criteria. During experiment, the alternating voltage of the exactly defined frequency from the synthetizer applied on the measuring circuit with crystal unit as a chemo- or biosensor. The impedance is scanned near the resonance frequency. In this way, it is possible to observe simultaneously the changes of viscosity as well as of surface mass. The required equipment (impedance and network analyzer providing suitable resolution) is very expensive [4]. The slower measurement (scanning of impedance curves in 10 sec) is satisfactory for most biochemical interactions.

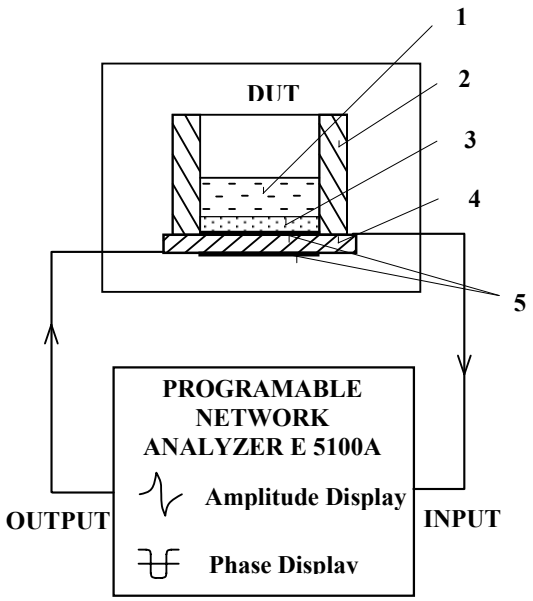


Fig. 1 Block diagram of a laboratory biosensor measuring systems. 1 - Bioactive liquid, 2 - Teflon cell, 3 - Buffer, 4 - AT quartz resonator in measuring π -network, 5 - Au electrodes.

## IV. Biochemical affinity interactions

An important issue affecting sensor performance is the proper attachment of a biological film to the sensor surface. Several immobilization techniques have been developed and its properties studied [3] to create an affinity sensor. For an affinity sensor, receptor molecules have to be immobilized on surface on the gold electrodes of the quartz disc. The area of the electrodes dimensions is characterized by maximum vibrations (trapped energy case). The corresponding binding molecule, if present in the sample solution, will bind specifically to the receptor of the surface. In this case the sensor directly responds to the formation of the receptor-ligand complex. When the ligand and receptor are an antigen and the relative antibody their interaction will lead to an immunocomplex.

We analyzed the binding between a covalently immobilized small molecule and its relative antibodies. Formation of immunocomplex AB at the surface of the quartz resonator vibrating in thickness-shear mode can be characterized by association $k_a$ and dissociation $k_d$ kinetic rate constants. The bindings curves of series resonance frequency $f_s$ vs. time $t$ were derived in [4]. The typical characteristics $f_s(t)$ of immunosensor shown three phases:

Relative stable background frequency $f_{s0}$ caused by presence of buffer (with their loading effect). 5 min typically.

Then, sample solution containing a mixture of antibody and analyte is injected (association phase). The antibodies with free binding sites interact with the immobiliyed ligand. A decrease of frequency $f_s$ is observed as a surface mass on the crystal increases (the equilibrium change $f_{eq}$ is achieved eventually). About 30 minutes.

Reaction of biosensor with phosphate buffer. Buffer is injected again and the dissociation of immunocomplexes is observed. From this phase, the dissociation constant $k_d$ can be obtained. The frequency $f_{sa}$ represents the amount of surface-bound immunocomplex at the beginning of dissociation. About 30 minutes.

Area of regeneration of biosensor after application of formic acid. 5 min typically.

Terminal phase of resonator regeneration. The process of regeneration of all covalent bonds between molecules of antibody and molecules of bioactive layer. About 5 minutes.

The reactions times depend on the concentration of antibody and analyte.

## V. Experimental results and discussion

### *A. Samples preparation*

The Y-cut quartz resonators as plates of different orientations were prepared. The Y-cut (YXl)-35,2° plates were prepared and electroded by Krystaly a.s., Czech Republic, and measured in our laboratory. The plates were 10 mm in diameter and 0.3 mm thick. The gold electrodes of 5 mm diameter were deposited on the major surfaces of the plates. The orientation of the plates was measured to ± 0.1°.

The main resonator parameters for the unloaded samples were measured under control voltage level $V_k$ = 0.2 $V_{RMS}$ using a network analyzer AGILENT E 5100A and measuring π-network 41900A. The linear electrical equivalent circuit parameters of the resonators were determined using a Saunders Test Temperature Chamber and 250 VP Transmission System.

The surface of the gold-electroded resonator was first treated 30 min with 100 ml of acetone, and dried. The second step is the activation process:

using cystamine solution ( 200 mg) in water, applied on the gold electrode surface (100 µl), incubated 2 hours, then crystal unit was washed with water and air dried, or

using glutaraldehyde 3% solution 3mg/97mg in phosphate buffer with pH=7.0, applied on the gold electrode surface (100 µl), incubated 1 hour, then crystal unit was washed and air dried, or

using albumin solution in phosphate buffer 500 µg/1ml, applied on the gold electrode surface (100 µl), incubated and stored at 4 °C 12 hours, then crystal unit was washed air dried.

The piezoelectric unit with biocomplex as biosensor is prepared to bind with its covalent layer the antibody liquid. The frequency shifts caused by activation process are given in Table 1.

TABLE I
THE CHANGES IN RESONANCE FREQUENCY CAUSED BY DIFFERENT MODE OF ACTIVATION.

| Resonator | $f_s$ [Hz] in measuring cell | $f_s$ [Hz] without cell |
|---|---|---|
| Without active surface | 5 036179 | 5 037956 |
| After treatment by acetone | 5 036660 | 5 038181 |
| After activation by cystamine | 5 036 499 | 5 038121 |
| After activation by glutaraldehyde | 5 036865 | 5 038659 |
| After activation by albumine | 5 037030 | 5 038962 |

The reference temperature is 25°C, the $f_s$ is series resonance frequency of fundamental harmonics ($n$ = 1).

### *B. Resonance frequency vs. time characteristics*

Using the passive method for determination of the resonance frequency and precise AGILENT E5100A, the affinity process was measured. In the Fig. 2 and 3 the antibody solutions ASC with phosphate buffer 1:100 and 1:250 respectively were used.

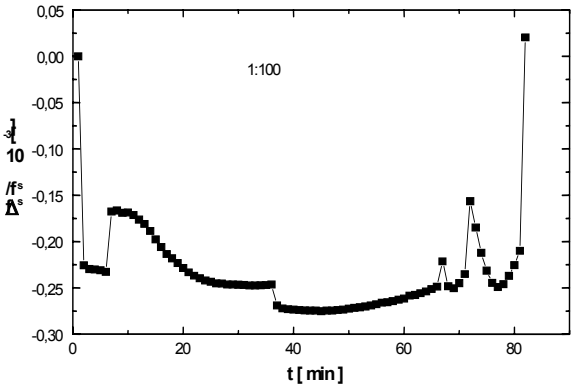


Fig. 2 Affinity reaction of the biosensor with the antibody ASC 1:100

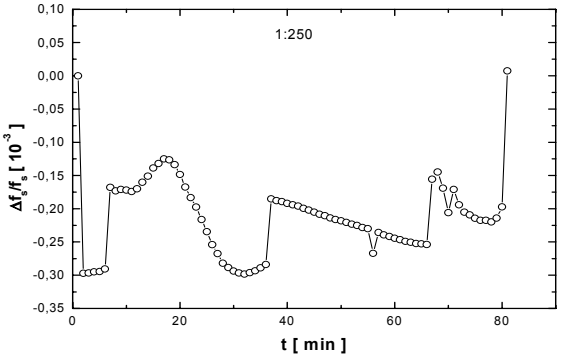


Fig. 3 Affinity reaction of the biosensor with the antibody ASC 1:250

The characteristics frequency-time correspond with the similarly cases published previously. The small differences in the characteristics described as a), c) and e) are caused by the process of regeneration, realized previously.

The attention should be devoted to the viscoelastic interaction of liquid and resonator surface, roughness of resonator surface, controlling electronic circuit, surface tension, and different chemical interaction of resonator surface - liquid.

The precise technique gives good possibilities for determination of more complicated cases of

biosensor´s interaction with bioactive materials, which are important in health treatment.

## VI. Conclusion

The possibilities or real time monitoring of the affinity – immunoreactions in the liquid phase were shown. The antibody – based or immunosensors have been the most explored and the most advanced class of biosensors. The interest results from the fact that antibodies can be obtained against almost any substance, antibody immobilization techniques are numerous. Other advantages of piezoelectric immunosensors are that they are reusable with no noticeable degradation in performance.

## Acknowledgment

This work was supported in part by the Ministry of Education of the Czech Republic under Contract Code MSM 242200002.

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