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Detection of flexibly bound adsorbate using the nonlinear response of quartz crystal resonator driven at high oscillation amplitude

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ABSTRACT

Flexibly bound heavy adsorbates as antibodies, biomarkers, microbeads, bacteria do not produce response at quartz resonators accounted by their mass alone, which complicates their acoustic detection. To resolve this problem, an anharmonic detection technique (ADT) has been developed. It generates higher harmonics by applying a high voltage (0.7–10 V) fundamental frequency excitation to 14.3 MHz AT-cut quartz crystal. Due to non-linearity the parameters of generated oscillations depend on the amplitude A of the applied signal, being proportional to A^2 for the fundamental resonance frequency and mainly to A^3 for the amplitude of generated 3rd harmonics. The coefficients of proportionality depend on number of attached particles, but not on their mass and can be calculated from the phenomenological model based on Duffing equation with damping. The model was tested in liquids of various viscosity, on composite layer including polymer film with deposited gold nanoparticles and in electrochemical mode for *E.-coli* adsorption.

1. Introduction

Typical application of quartz crystal microbalance either in resonance (QCM) or dispersive (network analyser) or mixed mode (QCM-D) implies using small amplitude oscillations arising at sinusoidal voltage of about 10 mV [1]. In this case the system oscillates within harmonic range and consequently a nearly linear response is observed, which means that the signal is recorded in the same frequency range as excitation with small corrections due to mass, viscosity, surface roughness, mechanical stress. It is interesting to see what will happen if sinusoidal excitation of large amplitude is applied, in the range of 0.1–10 V. One may expect that this excitation drives oscillations beyond purely harmonic range [2]. Anharmonicity leads to excitations on higher resonances. They are mainly determined by the quartz crystal itself. However, if there is an adsorbate on the surface, there may be contribution from adsorbate-substrate interactions which alters the non-linear response of QCM [3,4]. One can expect that if adsorbate is heavy enough and not rigidly bound, it may not "follow" the oscillations. Therefore the parameters of induced signal may reveal the characteristic of adsorbate – substrate bond, rather than simply attached mass.

With this in mind, anharmonic detection technique – instrument which serves as actuator of higher harmonics through applying of large amplitude excitation of fundamental frequency was designed and built. The description of the instrument and biological applications of the technique will be given in a future article. This paper deals with the physics of the method: description of anharmonically generated third and higher harmonics and the role of viscosity of adjacent liquid as well as parameters of the signal in the case of a thin viscoelastic layer, or composite, present on the quartz resonator surface. Also electrochemical version of the technique is presented, where high voltage generation is applied simultaneously with electrochemical potential. This method was tested on model system as *E. coli* bacteria attached to gold.

1.1. The phenomenological model for non-linear response

The phenomenological model of non-linear response in the presence of adsorbate can be developed basing on Duffing equation

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for anharmonic oscillator with driving force:

$$\ddot{x} + 2\lambda\dot{x} + \omega_0^2 x = A\cos\omega t - \beta x^3 + \frac{NS}{M_q}F_x \quad (1)$$

where x is displacements in the crystal (as shown in Fig. 1), ω_0 is undisturbed "linear" resonance frequency before binding, λ is viscous friction, β is non-linearity coefficient. The anharmonic term proportional to x^4 producing the same contribution as x^3 is disregarded because of symmetry considerations [5]. There are 2 external forces, one from oscillator driving voltage (normalized per M_q defined below) of amplitude A and frequency ω close to resonance ω_0. Another force along the quartz surface F_x is due to the presence of bound particles, its total value is obtained by multiplication by number of particles N per unit of surface, surface area S and divided by M_q, the nodal mass of the crystal, which is equal to half of the total mass. The movement of attached particle with mass m_s and proceeds parallel the surface, since it is considered to be bulky and cannot approach the surface due to geometrical constraints. Introducing the coordinate of the particle y, point of attachment on the surface x, bond length l, angle between the bond and surface θ, elasticity constant k, one can write particle motion under the Hook force projection, along the surface $F_x = -k\ \delta\ l\ cos\theta$ at small displacements $|x\text{-}y| << l$:

$$m_s\ddot{y} = -F_x = \frac{k(x-y)}{\sqrt{(x-y)^2 + l^2}}\left(\sqrt{(x-y)^2 + l^2} - l\right) \approx \frac{k(x-y)^3}{2l^2} \quad (2)$$

Furthermore, for a heavy particle, so that $m_s >> k/{\omega_0}^2$, from (2) $\ddot{y} \approx 0, y \approx 0$. Therefore the particle is immobile in laboratory frame and the force F_x in Eqs. (1) and (2) is $F_x = -k\ x^3/2l^2$. Upon substitution of this force the equation of anharmonic motion of quartz with flexibly attached heavy particles reads

$$\ddot{x} + 2\lambda\dot{x} + \omega_0^2 x = A\cos\omega t - \beta x^3 - \frac{NSkx^3}{M_q 2l^2} \quad (3)$$

Thus, the assumption that adsorbate is flexibly bound, which for heavy particle means that it is immobile in the laboratory frame, leads to anharmonic force acting upon surface, proportional to Hooke constant. Since no assumptions regarding anharmonicity of bond have been made, the Eq. (3) is quite general. The fact that this anharmonic force is proportional to the number of particles provides foundation of the method of measuring of their surface concentration by using induced anharmonic quartz oscillations. Absence of movements of adsobate allows to disregard hydrodynamic flow acting on particles, and to consider liquid only acting on quartz surface, as will be shown below.

All terms in the right hand, except external force are considered to be small. In this case the iterative solution in series is well known [5]. It is main resonance $a\cos\omega t$ on frequency shifted from fundamental and third harmonics $a^{(3)}\cos 3\omega t$. The equation for amplitude a of the main resonance is

$$a\sqrt{(\omega - \omega_0 - \varkappa a^2)^2 + \lambda^2} = \frac{A}{2\omega_0} \quad (4)$$

where parameter x is expressed in terms of anharmonic constant of quartz and parameters for adsorbate.

$$\varkappa = \frac{3}{8\omega_0}\left(\beta + \frac{NS\ k}{M_q 2l^2}\right) \quad (5)$$

Next iteration gives rise to the amplitude $a^{(3)}$ of the third harmonics.

$$a^{(3)} = \frac{A^3\varkappa}{96\omega_0^4\left(\left(\omega - \omega_0 - \frac{\varkappa}{4\omega_0^2\lambda^2}A^2\right)^2 + \lambda^2\right)^{\frac{3}{2}}} \quad (6)$$

The dependence of the driving force on the amplitude A is mainly cubic. The resonance is shifted by the value dependent on A. The loss constant λ is expressed in terms of experimentally accessible parameters as motional resistance R and inductance L of equivalent circuit, and quality factor Q, as follows:

$$\lambda = \frac{R}{2L} = \frac{\omega_0}{2Q} \quad (7)$$

Motional resistance is the sum of motional resistance of quartz itself R_0, and R, the real part of acoustic impedance Z. In case of semi-infinite one-sided liquid contact:

$$R = ReZ_L = \sqrt{\frac{\rho\omega\eta}{2}} \quad (8)$$

where the liquid has viscosity η and density ρ. In case of finite viscoelastic layer, covering the quartz crystal and contacting liquid from the outside, the real part should be taken of the total acoustic impedance, which is [2]:

$$Z = Z_P\left(\frac{Z_L\cosh(\gamma h_f) + Z_P\sinh(\gamma h_f)}{Z_P\cosh(\gamma h_f) + Z_L\sinh(\gamma h_f)}\right) \quad (9)$$

Eq. (9) describes the acoustic impedance for fundamental oscillations which was used in our previous work, where $\gamma = i\omega(\rho_f/G)^{1/2}$ is the shear wave propagation constant and $G = G' + iG''$ is the complex shear modulus of the film of thickness h_f. $Z_P = (\rho_f G)^{1/2}$ is the acoustic impedances of the film (of density ρ_f).

The Eq. (6) shows that amplitude of generated harmonics depends on number of flexibly attached particles. It is essential that driving oscillations are applied to fundamental mode only. Therefore, if one can build an instrument which generates and records higher harmonics, then the concentration of weakly bound adsorbate can be measured. This instrument is described further on.

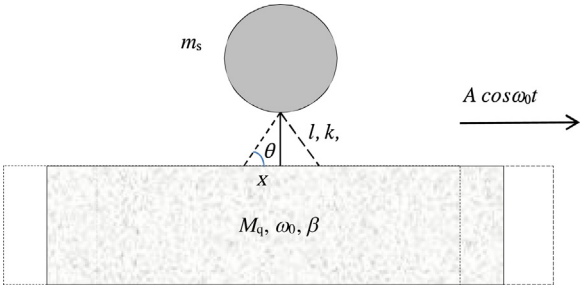

Fig. 1. Schematic presentation of the model. Analyte of mass m_s is attached to quartz of nodal mass M_q with a bond with length l, harmonic constant k. The quartz has frequency ω_0 and anharmonic constant β. Displacements in quartz x are caused by external force $A\cos\omega_0 t$, with frequency close to fundamental frequency ω_0. Movements of heavy attached particle are negligible. Because of its inertia it effectively pulls the crystal back.

2. Experimental

Anharmonic detection technique (ADT) allows measurements of response on fundamental resonance and higher harmonics to the signal of varied amplitude applied to fundamental resonance only. Therefore it is a development of network analyser, into actuator. Two modes of measurements were used. In the first one frequency was scanned around resonance, which was about 14.3 MHz (AT-cut crystal, Laptech) at constant amplitude. The frequency span could be varied between 0 and 200 kHz. The applied amplitude was varied with the highest limit up to 10 V which is significantly higher than typical network analyser applied amplitude of 10–20 mV. This allowed the significant anharmonic