

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

Shilpa Khobragade¹, Samantha Swarbrick¹, Violeta-Tincuta Gruid², Igor Efimov^{3}, Adriana Ispas², Victor Ostanin⁴, Andreas Bund², Sourav Ghosh¹*

¹Centre for Biological Engineering, Loughborough University, Holywell Park, LE11 3TU
Leicestershire, UK

²Electrochemistry and Electroplating Group, Technische Universität Ilmenau,
Gustav-Kirchhoff-Str. 6, 98693 Ilmenau, Germany

³Interdisciplinary Biomedical Research Centre, Nottingham Trent University, Clifton Lane,
Nottingham, NG11 8NS, UK. Corresponding author. Email: igor.efimov@ntu.ac.uk

⁴Department of Chemistry, Cambridge University, Lensfield Road, Cambridge, CB2 1EW, UK

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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 (0.7-10V) 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 0.1-10V. 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 for anharmonic oscillator with driving force:

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

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 $|x-y| \ll l$:

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

Furthermore, for a heavy particle, so that $m_s \gg k/\omega_0^2$, from (2) $\ddot{y} \approx 0, y \approx 0$. Therefore the particle is immobile in laboratory frame and the force F_x in eqn (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

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

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 eqn 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 adsorbate 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

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

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

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

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

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

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:

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

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 equation (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.

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 14.3MHz (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 10V which is significantly higher than typical network analyser applied amplitude of 10-20 mV. This allowed the significant anharmonic generation. For convenience, the experimental data will be reported in terms of normalized units, so that 1 unit on applied voltage scale corresponds to 30 V.

In the second mode, the frequency of applied signal was constant, but its amplitude was linearly increased with time from 0 up to 10V. This method has certain advantage at large amplitude, because it allows avoiding heating of the crystal. In the both cases the amplitude of generated 3rd harmonic was analysed by applying Eq (6), where either amplitude A or frequency ω were fixed. The experiments were carried out either in a microfluidic flow-cell, or in an electrochemical cell. The former was used for measurements on bare crystal in methanol and water. The latter was used for anharmonic measurements on polymer layers with or without Au nanoparticles on top of it.

For polymer deposition experiments QCM Au electrode was cleaned by brief immersion into 30% nitric acid. The polymer used, poly (3,4-ethylene-dioxythiophen) (PEDOT) was deposited potentiostatically from 30 mM EDOT monomer in 0.1 M LiClO₄ at 0.9V (Ag/AgCl in 3 M KCl) for 9 min⁶. The process was monitored by QCM measurements and led to 0.6 μm thick film. The solvent was changed to 0.5M H₂SO₄, in which the potential of the working electrode was cycled between 0.3 and 0.7 V at 50 mV/s. Subsequently, the polymer was reduced in the same solution at constant potential E=-0.5 V for 610 s. 1mM HAuCl₄ solution was injected in the H₂SO₄ electrolyte and the open circuit potential was monitored. The formation of gold nano-clusters on PEDOT surface occurred spontaneously and the open circuit potential increased up to 0.7 V in 10 min. Then the solvent was changed to 50 mM phosphate buffer. The ADT signal was measured at various stages of this procedure without applied potential.

Potential-dependent ADT was carried out as follows. Potentiostat was used in a circuit with working electrode on virtual ground. High voltage from ADT instrument was applied to outer electrode of QCM, whereas facing electrolyte electrode was on common zero. This allowed avoiding disturbance of current in the electrochemical cell. Nonlinear potential-dependent signal was studied in PBS on Au surface functionalized with Ecoli, which provided model system for flexibly bound heavy adsorbate. In this experiments Au surface was prepared by cleaning quartz crystals in acetone, isopropyl alcohol (IPA), ultra-pure 200-proof ethanol (Sigma-Aldrich, UK) and ultrapure water (Milli-Q) were used. Functionalization was done by depositing on gold substrate biotin terminated thiol, followed by streptavidin and biotinylated Ecoli aptamers. Thiols with biotin terminal group HS-C11-(EG)6-Biotin (th004-m11.n6-0.005) and methoxy HS-C11-(EG)3-OCH₃ terminal group (th006-n3) were obtained from Prochimia Surfaces, Poland. The content of biotin terminated thiol was 1%. The biotinylated ssDNA aptamer (88-mer)

targeting *E.coli* bacteria strain (KCTC 2571) with the sequence: 5'-GCAATGGTACGGTACTTCCA CT TAGGTCGAGGTTAGTTTGTCTTGCTGGCGCATCCA CTGAGCGCAA AAGTGCACGCTACTTTGCTAA-3' was used. *E.coli* (KCTC 2571) strain was procured from Microbial Resource Center / Korean Collection for Type Culture (KCTC), Korea Research Institute of Bioscience and Biotechnology. Freshly grown Ecoli in concentration of 10^7 1/cm³ were flown on top of this layer. Specially redesigned flow cell, 70 μ l volume, with inlet for reference Ag/Ag Cl microelectrode and Pt wire was used in these experiments. ADT signal was compared before and after Ecoli injection.

3. RESULTS

3.1 Verification of the model for liquids of different viscosities.

To prove the validity of the model eqn (1)-(9), the ramps of amplitude of oscillations ranging from 0 to 0.7 units (or to 2.8 V) applied to bare crystal in methanol and water. The driving frequencies $f (= \omega/2\pi)$ were chosen at resonance $f_0 (= \omega_0/2\pi)$ and around it: $f_0 -3, f_0 -2 \dots$ etc kHz. The amplitude of 3rd harmonics as a function of applied voltage is shown in Fig 2 a,b for methanol and water correspondingly. The fitting to the Eq (6) at N=0 since there is no attached particles, is shown on the plot as well. It was done as follows: parameters for the crystal in methanol at resonance were fitted only once. The other curves for methanol (Fig 2 a) were obtained at the same set of parameters, by shifting by -3.... +3 kHz off the resonance frequency f_0 . Changing to water leaves intact all parameters in Eq (6) except the loss parameter λ which depends on viscosity through Eqs. (7) and (8). The acoustic resistances for methanol and water were measured to be 187 and 237 Ohm respectively. To these values the acoustic resistance of quartz should be added, which was 50 Ohm. Therefore, λ as fitted to methanol, was rescaled by

factor of $(237+50)/(187+50) = 1.21$. With this only adjustment the theoretical curves were plotted for water, as shown in Fig 2b. The agreement is good, which supports the model (1)-(9). Some deviation is observed at higher driving amplitude which is due to contribution of 5th term of solution $O(A^5)$.

The same set of data can be presented as a function of frequency at fixed amplitude driving force as shown in Fig 3 a, b for the crystal in methanol and water respectively each for 0.3 and 0.4 units. The experimental points are the same as in Fig 2 a, b taken at fixed amplitude and for various frequencies. The agreement with Eq. (10) is reasonably good for the whole range from $f_0 - 4\text{kHz}$ to $f_0+4\text{ kHz}$. The formula reproduces the asymmetry of the resonance curve due to non-linearity.

3.2 Verification for PEDOT layer and Au nanoparticles

Fundamental and third induced resonance spectra were taken for bare crystal in solvent, crystal with a layer of PEDOT of 0.6 μm , and PEDOT with Au nanoparticles deposited as described in experimental section. The thickness of the polymer layer and the presence of nanoparticles were analysed in the previous work [6], which conditions were repeated here. Figure 4 shows the dependence of amplitude of the third harmonics in resonance on the driving voltage. The lines show best fit to the Eq. (6) which in case of exact resonance acquires a simple form:

$$a^{(3)} = \frac{\kappa A^3}{96\omega_0^4 \lambda^3} \equiv CA^3 \quad (10)$$

where λ is related to the real part of acoustic resistance R by Eq(7). The last value is measured in impedance spectra for fundamental resonance. One can observe that a cubic dependence on applied voltage A holds for all curves. Equation (10) contains 2 parameters: κ as defined by

Eq(6) depends on intrinsic non-linearity of the crystal, β , and the number of loosely attached particles, N , oscillating out of phase. The denominator as stated above is proportional to the losses, or particles oscillating in phase with the motion of the crystal. From figure 4, it follows that the amplitude of the third harmonic decreases, from the bare crystal in water to the crystal with deposited PEDOT film. This corresponds to the increase of acoustic impedance due to the deposited polymer. The influence of loosely attached particles is small, though cannot be neglected. When Au nanoparticles were deposited on the top of the polymer layer, then the amplitude of the third harmonic increased. This corresponds to an increase of the parameter κ , or the number of particles N that oscillate out of phase.

For the same set of experiments the shift of the fundamental resonance frequency is shown in Fig 5 as a function of the applied voltage, A . Fitting is done according to Eq. (4), which in the vicinity of resonance in the first approximation reads:

$$\omega - \omega_0 = 2\pi\Delta f = \frac{\kappa A^2}{4\omega_0^2 \lambda^2} \equiv bA^2 \quad (11)$$

The dependence on voltage as A^2 reasonably holds for all 3 curves. One can note that the positive shift in frequency due to anharmonicity is quite noticeable, up to 600 Hz.

Similarly to the amplitude of third harmonic Eq. (11), the frequency shift is proportional to the parameter κ which accounts for loosely bound particles on the surface and is inversely proportional to the squared losses. Deposited polymer increases the losses compared to the bare surface, so the frequency shifts is smaller than in water (Fig 5). Added Au nanoparticles increase the parameter κ , which leads to larger frequency shifts. The fitting parameters, along with the acoustic resistances for all 3 surfaces are shown in Table 1. Since only the ratios of fitting parameters are of interest, they are shown in arbitrary (but the same) units.

Equations (10) and (11) are independent. Therefore they can be used as a test for the consistency of the model. From (10) and (11) one can calculate the ratio $\kappa_{pol} / \kappa_{polAu}$ for the data for the polymer and for the polymer with AuNP in terms of the fitted parameters C_{pol} , C_{polAu} or b_{pol} , b_{polAu} and acoustic impedances R_{pol} , R_{polAu} from the Table 1. From (10) it follows that:

$$\frac{\kappa_{pol}}{\kappa_{polAu}} = \frac{C_{pol}}{C_{polAu}} \frac{R_{pol}^3}{R_{polAu}^3} \quad (12)$$

whereas from (11):

$$\frac{\kappa_{pol}}{\kappa_{polAu}} = \frac{b_{pol}}{b_{polAu}} \frac{R_{pol}^2}{R_{polAu}^2} \quad (13)$$

For the model to be consistent, both equations should produce the same result. Substituting the data from Table 1 one can obtain 0.61 in (12) and 0.7 in (13), which is in a good agreement. However, for the ratio $\kappa_{sol} / \kappa_{pol}$ for solvent and polymer agreement is not perfect, with the values are 0.91 and 1.64 from (12) and (13) respectively.

3.3 Potential-dependent generation of 3rd harmonic with and without attached bacteria

Results of electrochemically controlled third harmonic generation are shown in fig 6. The amplitude of the ADT signal was measured as a function of potential of QCM Au electrode in PBS at static potentials between -0.1 and 0.6V/AgCl shown by points. In the first experiment, the surface of gold was functionalized with thio/streptavidin/Ecoli aptamer layer. Subsequently, Ecoli were flown in concentration of 10^7 cm^{-3} over the same surface and ADT signal was measured again at the same inducing voltage and static electrode potentials. The generated 3rd

harmonics is clearly potential dependent with minimum approximately at the potential of zero charge.

Bacteria bind to specific aptamers and create layer of flexibly bound heavy particles which should increase the ADT signal, according to the model. This is exactly the case, the signal in the presence of attached bacteria is 10-15% larger in the potential range outside of zero charge potential.

4. DISCUSSION

Positive frequency shifts, or anti-Sauerbrey behavior, due to the flexible attachment of various particles have been known for quite a long time. The examples include attachment to QCM of various materials such as: immunoglobulin G [7], salmonella [8], viruses on molecular imprinted polymers [9], glass spheres with different liquid contacts [10], TiO₂ nanocontainers and streptavidin coated magnetic microbeads [11]. In [12] the QCM response for a set of bioanalytes as almond protein, sesame protein, yeast cells, HeLa cells was studied for polymer films of different morphology. The anti-Sauerbrey frequency shifts were observed on not-matching surfaces such as porous polymers, flat polymers, i.e. where rigid binding did not occur. The model describing positive frequency shifts based on earlier work on coupled resonators [13] was given in [10]. There were no methods of measuring the QCM response at various amplitudes of oscillations, therefore the model is linear and the frequency shift independent of amplitude. However in [2], in the chapter on anharmonic effects in QCM it was correctly derived, that the frequency shift should be positive and proportional to the squared amplitude.

The outlined above area of observed effects is very wide – attachment of viruses, proteins, nanoparticles, microbeads on various types of surfaces as molecular imprinted polymers, porous

polymers, self-assembled monolayers. One can suggest that deeper understanding of how to detect flexibly bound species is necessary. For this purpose more sophisticated techniques than usual QCM or network analyser are necessary.

The introduction of the anharmonic detection technique [3,4] and especially its latest version used in this work allowed detection of smooth, noise-free responses at higher harmonics, up to 5th, induced by high amplitude excitation at fundamental frequency. Therefore, a new nonlinear model was developed for the interpretation of the experimental data. It uses ideas of coupled resonators, as in [10,13], with one complication and one simplification added: nonlinear terms were added but only the low frequency limit, or the case of infinite attached mass, was investigated. Rigorous theoretical treatment of nonlinear resonance in quartz plates, although without adsorbed mass, was provided by Tiersten [14].

Application of the anharmonic detection technique can be wider than just capturing flexibly bound adsorbate. The formula (6) for amplitude of generated 3rd harmonics by means of eqn (7), (8) contains acoustic resistance of the deposited layer in contact with liquid. This layer is treated in usual way, i.e. as non-flexible. The value of its acoustic resistance should be taken at main resonance. It is defined by losses and storage moduli of the film, which may be solvated depending on applied potential [15] in case of redox polymers. It also depends on the surface roughness of interface with liquid [16]. Therefore, theoretically one can correlate attachment of flexibly bound adsorbate with liquid content in the film and its and roughness.

More effects may arise from film resonance phenomenon, in which elastic parameters and thickness of the film are such that it sustains its own resonance at fundamental frequency [17]. Anharmonic generation of third harmonics in this case as well as movement of flexibly bound

adsorbate is quite complex computational problem, which may result in new experimental approaches.

Of course the model has its limitations. For example the χ_{sol} / χ_{pot} calculated from frequency shift and amplitude do not match well for bare crystals in water and crystals with polymer layers in contact with water. Obviously the polymer layer cannot be considered as flexibly attached infinite mass. However the same ratio calculated for gold nanoparticles deposited on top of the polymer is much better. Also the model describes correctly the nonlinear effects in case of contact with bulk liquids of various viscosities.

The results of electrochemically-controlled ADT experiments can be rationalized in terms of Lippmann equation. Generation of the third harmonics depends on surface tension of gold in electrolyte: the higher surface tension, the lower conversion of fundamental oscillations into higher harmonics. Lippmann equation surface tension σ of an electrode on electrode potential in the presence of adsorbate with concentration c reads [18]:

$$d\sigma = -qd\varphi - RT\gamma d\ln c$$

R is gas constant, T temperature, γ is the maximal adsorption. In the absence of any adsorption, the equation states that surface tension reaches maximum at potential of zero charge. This is supported by fig 6, where the amplitude of third harmonics is minimal at $E=0V/AgCl$, which corresponds to potential of zero charge for Au electrode in PBS. Addition of Ecoli leads to shift of potential of minimum generation to 0.1 V for 10^7 cm^{-3} Ecoli. The shift is defined by the second term in r.h.s. of Lippmann equation. Exact analysis requires a model for dependence of electrode charge on q . The advantage of third harmonics generation measurements is clearly seen in increased amplitude of ADT signal at potentials outside the zero charge.

5. CONCLUSIONS

An anharmonic detection technique for excitation of higher harmonics by application of fundamental one of varying amplitude is tested for QCM contacts with liquids of various viscosities, polymer and composite layers loads. To describe experimental findings the analytical model is developed based on the Duffing equation. The positive shifts of resonance frequency proportional to A^2 and amplitude of the generated 3rd harmonic proportional to A^3 agree with calculations. Parameters of shifts allow the detection of flexibly bound heavy particle which has wider possibility of applications. Electrochemically controlled ADT shows potential dependence with minimum at zero charge but increasing in both cathodic and anodic. This can be used for sensing purposes.

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FIGURE CAPTIONS

Figure 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.

Figure 2 a,b. Dependence of amplitude of generated 3rd harmonics on amplitude of applied signal of fundamental frequency for QCM in methanol (a) and water (b). The frequency of applied signal was shifted with respect to resonance as indicated. Solid lines show experimental data. Dashed lines show best fit to the Eq (6). Fitting was done only once for methanol at one frequency. All other curves for methanol and all for water were calculated by substituting different frequencies and viscosity while keeping fitted parameters the same.

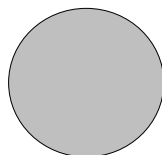
Figure 3 a,b. Points show experimental data from Figure 2 a,b correspondingly at fixed applied amplitude as indicated and varying frequencies. Solid lines show calculation according to Eq (6).

Figure 4. Dependence of amplitude of 3rd harmonics on amplitude of applied signal of fundamental frequency for contact with water of: bare crystal (designated as “sol”), crystal with PEDOT layer (“pol”) and crystal with PEDOT layer and deposited gold nanoparticles on top of it (“polAu”). Experimental data are shown by points, fitting to Eq (10) in the text is shown by lines as indicated in the figure.

Figure 5. Shift of fundamental frequency (with offset to zero) as a function of amplitude of applied signal of fundamental frequency for experiment in Figure 4.

Figure 6. Amplitude of 3rd harmonics as a function of electrode potential on QCM Au electrode functionalized with thiol/streptavidin/Ecoli aptamers layer before and after flowing of 10^7 cm^{-3} Ecoli.

FIGURES



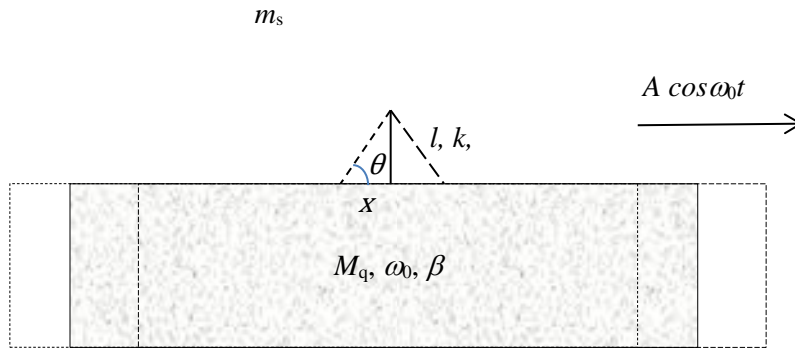
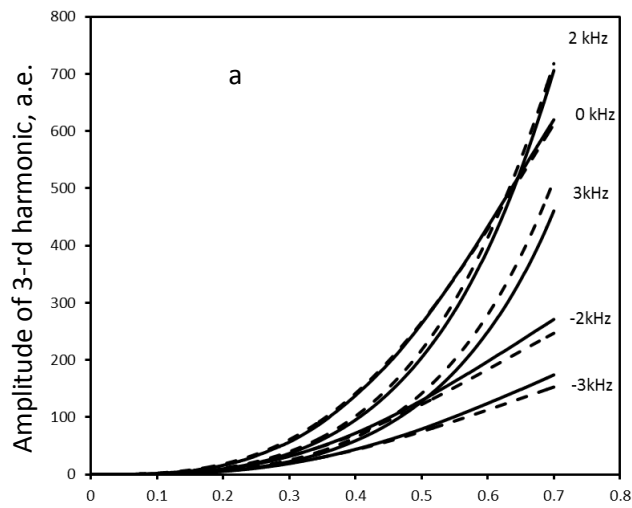


Figure 1



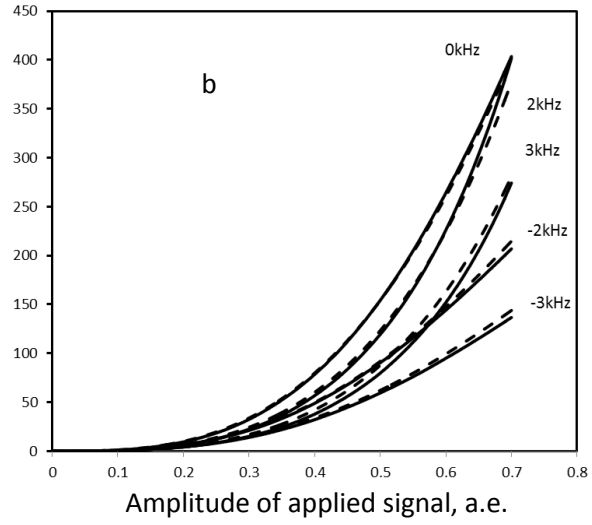
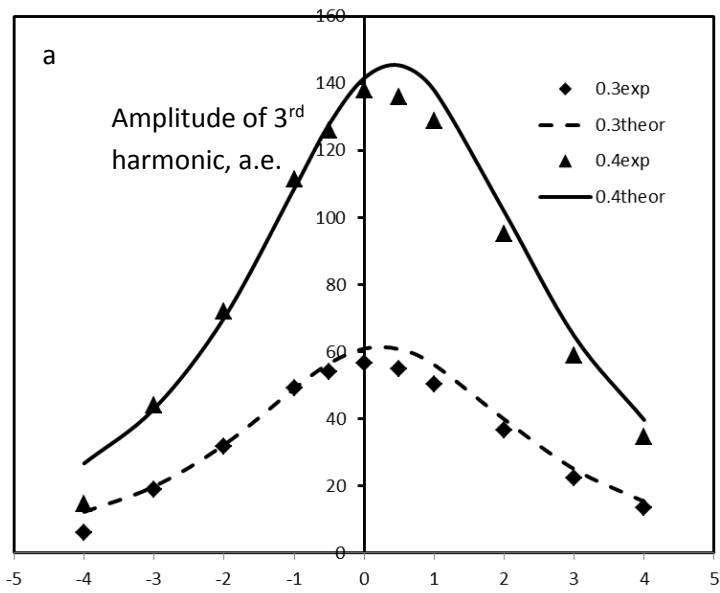


Figure 2a,b



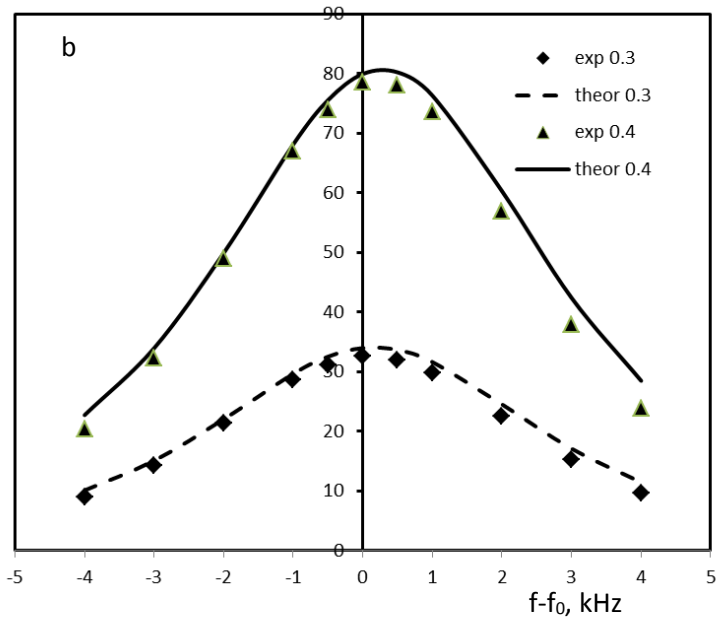


Figure 3 a,b

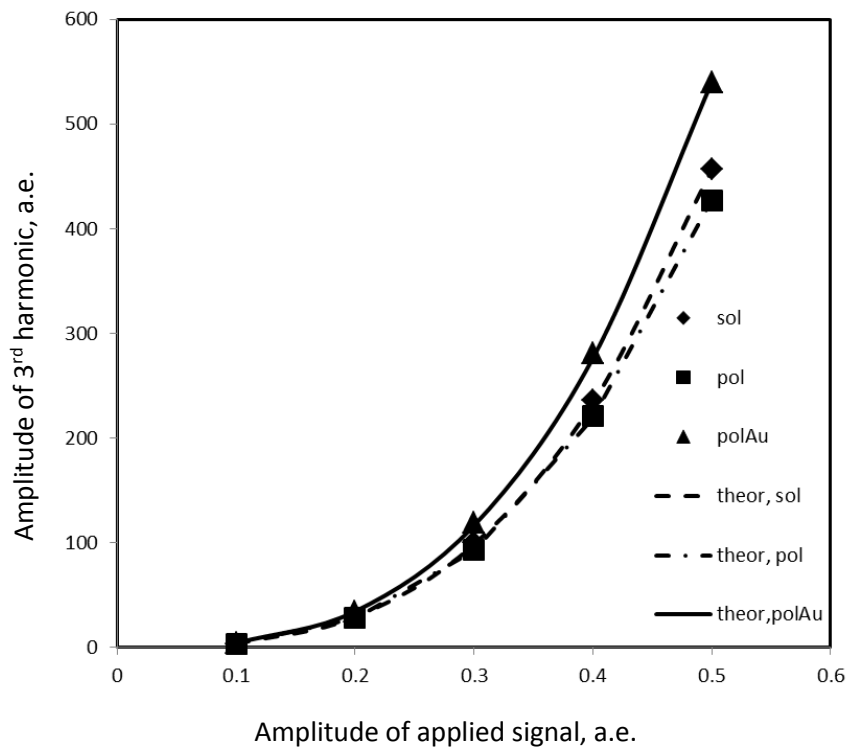


Figure 4

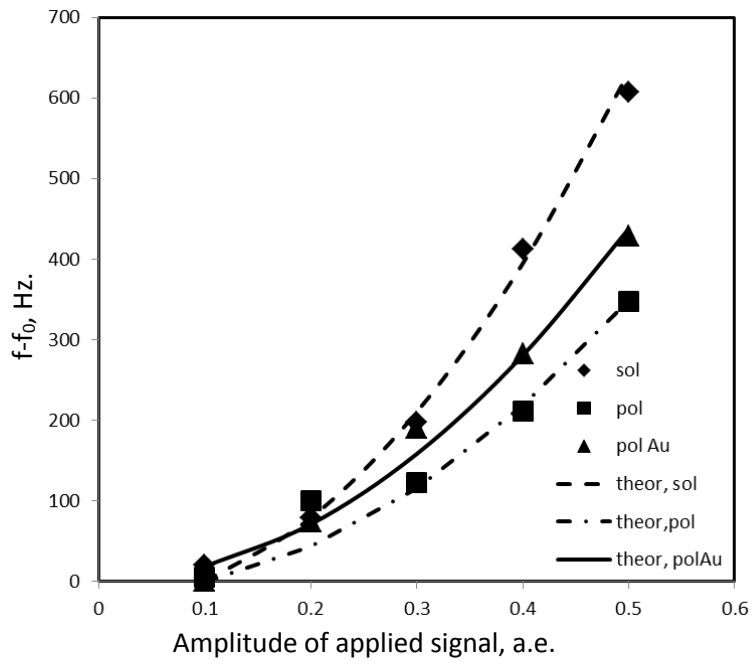


Figure 5

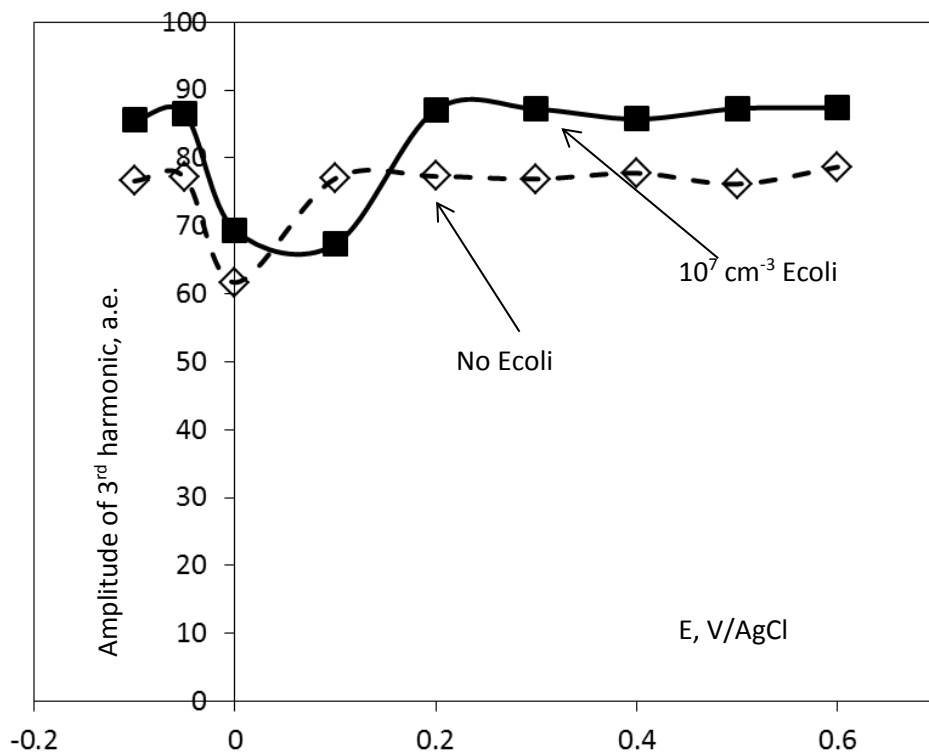


Figure 6.

TABLE

parameters interface	C , fitted from (11)	b , fitted from (12)	R , Ohm
solution	3.65	2.64	163
polymer	3.41	1.45	172
polymer AuNP	4.32	1.74	188

Table 1. Parameters C and b obtained by fitting of Eqs (10) and (11) to experimental data fig 4 and fig 5 respectively. R shows real part acoustic impedance in resonance. The data are shown for bare crystal in solution, crystal with deposited PEDOT, and crystal with deposited PEDOT and Au nanoparticles.

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