

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D): Real-Time Characterization of Nano-Scale Interactions at Surfaces

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ABSTRACT

There has been an increasing demand for analytical tools to quantify the interactions and/or reactions of nano-scale particles, polymers and bio-molecules, with a variety of surfaces, in real time. Understanding the behavior of such molecules at the nano-scale enables researchers to optimize the conditions for desired results at macro scale.

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D), a nanomechanical acoustic-based analytical technique, provides in-situ, real-time characterization of materials at interfaces. The present report is focused on applications of the QCM-D technique in real-time characterization of Au/Ag nanoparticle adsorption, and the interaction of various biomolecules such as proteins, DNA, and viruses with other biological materials, polymers or inorganic surfaces.

Keywords: QCM-D, Nanoparticles, Polymers, Protein, DNA.

INTRODUCTION

Quartz crystal microbalances (QCMs) have been used for decades to monitor the growth of thin films in vacuum. More recently, invention of QCMs that operate in liquid has opened up numerous possibilities of applying this technique in the fields of nanotechnology, biotechnology, biology, drug delivery, etc., where real time, in-situ analysis of interactions of nano-scale molecules/particles at various surfaces, in a liquid medium, is of critical importance.

A QCM consists of a thin quartz disc sandwiched between a pair of electrodes. Due to the piezoelectric properties of quartz, these crystals can be excited to oscillate at their resonance frequency by applying an AC voltage across the electrodes. In the event of any adsorption onto the surface of the oscillating quartz crystal, the frequency decreases. The resonance frequency (F) of the crystal depends on the total oscillating mass, including water coupled to the oscillation.

For a thin and rigid film, the decrease in frequency is linearly proportional to adsorbed mass, defined by the Sauerbrey relation¹:

$$\Delta m = -\frac{C \cdot \Delta f}{n} \quad C = 17.7 \text{ ng Hz}^{-1} \text{ cm}^{-2}$$

$n = 1, 3, 5, \text{ etc.},$ is the overtone number.

When the adsorbed film is not rigid, the Sauerbrey relation becomes invalid. By measuring multiple frequencies and the energy dissipation of the quartz sensor it is possible to determine if the adsorbed film is rigid or soft. In contrast, conventional QCMs only measure changes in frequency and hence fail to fully characterize a soft material.

With QCM-D, simultaneous measurements of changes in resonance frequency (ΔF) and energy dissipation (ΔD) are performed by periodically switching off the driving power to the sensor crystal and recording the decay of damped oscillation as the adsorption and/or structural changes take place (Figure 1). While changes in frequency (ΔF) provide information about mass changes, changes in dissipation (ΔD) provide structural information about the viscoelastic properties of adsorbed films in real time.

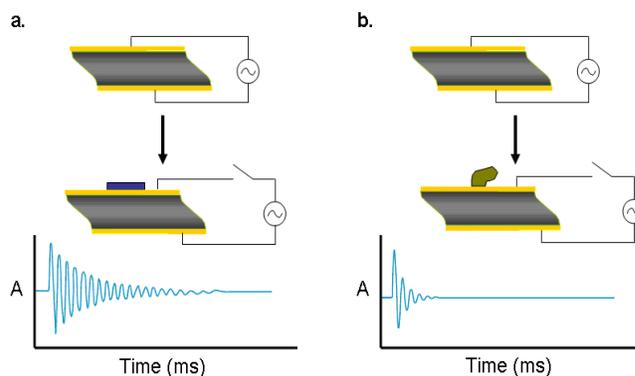


Figure 1. Cartoon depicting the viscoelastic differences between (a) a rigid material and (b) a soft viscoelastic material. In each case, both the change in frequency (change in mass) and change in dissipation (change in viscoelasticity) are monitored simultaneously

Figure 2 represents typical frequency (ΔF) and energy dissipation (ΔD) changes vs. time plot for a protein-protein interaction. By measuring ΔF s and ΔD s at multiple frequencies and applying a viscoelastic (Voigt) model, the adhering film can be characterized in detail and viscosity, elasticity and the correct thickness of the adsorbed films may be extracted.

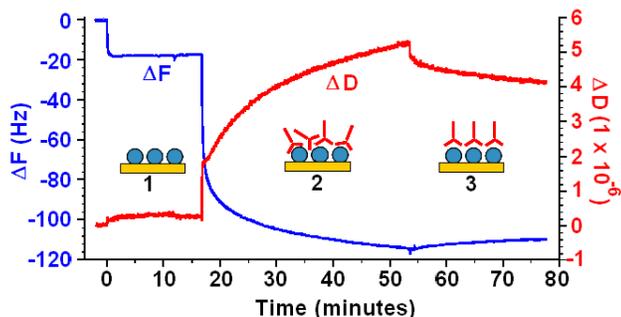


Figure 2. Frequency change (ΔF) and dissipation change (ΔD) vs. time plot for the reaction of a globular protein e.g. antigen with elongated protein e.g. antibody. Step 1: adsorption of antigen on to the crystals surface, Step 2: reaction of antibody with the adsorbed antigen molecules, Step 3: rinsing of loosely bound molecules and reorganization

MONITORING NANO-SCALE INTERACTIONS AND REACTIONS BY QCM-D

Applications of QCM-D can be divided into two broad categories:

1-Surface interactions: Interactions between nano-scale molecules and surfaces as well as interactions between various molecules on a surface are studied. Examples include: nanoparticle-substrate interactions, biomolecular interactions (protein, DNA, antibody), polyelectrolyte multilayer buildup, enzyme-substrate interactions, etc.

2- Surface reactions: Reactions within the adsorbed layer on the surface are studied. Examples include: cross-linking of proteins, polymers, conformation changes, hydration/swelling of polymers and other thin films, etc.

This article describes the results of a recent QCM-D experiment carried out to study the adsorption of Au/Ag nanoparticles, both in static and dynamic modes, onto SiO₂ surfaces.

We also present other examples of QCM-D applications that have been reported elsewhere in detail. Specifically the study of Ti-specific Phage display viruses, buildup of polyelectrolyte multilayers and DNA-PNA hybridization are briefly described here.

1 STUDY OF CHARGED NANOPARTICLE ADSORPTION

In an earlier study it has been established that surface adsorption of oppositely charged nanoparticles is driven by cooperative electrostatic interactions and does not require chemical ligation or layer-by-layer schemes². The composition and quality of the coatings can be regulated by the types, the charges, the relative concentrations of nanoparticles and the pH.

In the present study, we have investigated the effect of static and dynamic motion of charged nanoparticles solutions on their adsorption on silica surfaces.

1.1 Experimental

Materials: Silicon dioxide-coated 5-MHz quartz sensor crystals were cleaned using the following protocol: UV/ozone treatment for 10 minutes followed by cleaning in a 2% SDS solution for 30 minutes and rinsing with DI water and thereafter UV/ozone treatment for another 10 minutes.

Three concentrations of 1:1 Au/Ag nanoparticle slurries; 50 $\mu\text{g/ml}$ and 150 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$ in DI water were used. A mercaptoundecanoic acid coated Au nanoparticles (AuMUA) (2 mM) slurry was used as a non-coating control solution.

Methods: The experiment was carried out on a Q-Sense E4 instrument. An E4 sample chamber has four removable flow modules, each holding one sensor. In the present study, all four flow modules containing silicon dioxide coated crystals were used in a parallel configuration. In the first three modules, solutions of coating nanoparticles (1:1 Au/Ag); 50 $\mu\text{g/ml}$, 150 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$ respectively, were pumped. The fourth module was used as a control, where solution of non-coating nanoparticles (AuMUA) was pumped. Such parallel configuration of flow modules enabled four parallel, real time and simultaneous measurements of frequency (F) and dissipation (D) changes for different concentrations of nanoparticles. All the measurements were performed at 25°C, and under both static and flow modes of nanoparticles solution. A peristaltic pump was used to give a flow of 50 $\mu\text{l/min}$.

ΔF and ΔD plots were recorded at 5 overtones (3rd 5th 7th 9th and 11th) and fundamental frequency (5 MHz) of crystal sensor. For clarity reason, data for only the third overtone (15MHz) are shown in the present report.

1.2 Results and discussions:

Deposition of 1:1 Au/Ag nanoparticles on the silica surface began as soon as the solutions were introduced into

the flow modules. Continuous increase in the adsorbed mass on the crystal due to deposited nanoparticles induced a continuous decrease in frequency as shown in figure 3.

Dissipation change of these crystals showed an interesting behavior; a small but sharp increase in ΔD was observed as the nanoparticle layer begins to form (Inset Fig 3), which then approaches its original value relatively slowly. This result indicates that initially, the nanoparticle layers are soft (represented by increase in dissipation) with some water entrapped within clusters. As more particles are added, the rigidity of these films increases which is represented by a decrease in dissipation

Addition of non coating AuMUA nanoparticles did not show any shift either in frequency or dissipation indicating that these nanoparticles did not adsorb onto silica surface. Each sensor was subsequently evaluated in a scanning electron microscope (SEM) and all micrographs correlated with the QCM-D results (data not shown).

Adsorption in flow mode and static mode: effect of concentration:

Figure 3 represents a comparison between the frequency and dissipation responses of three crystals that will be referred to as 1, 2, and 3, on exposure to 50 $\mu\text{g/ml}$, 150 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$ respectively.

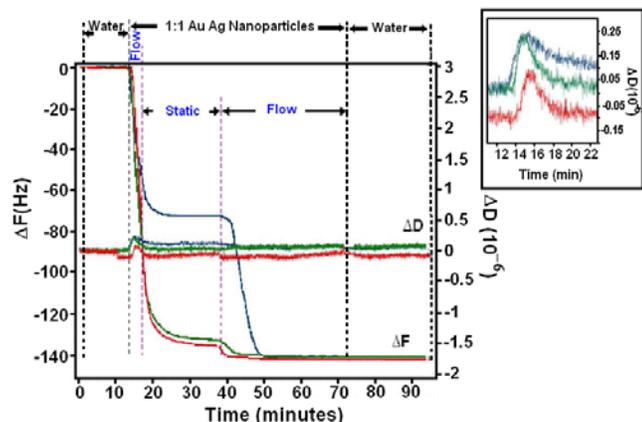


Figure 3. Frequency and dissipation responses, at the 3rd overtone of crystal 1, 2, and 3 on exposure to 50 $\mu\text{g/ml}$ (blue lines), 150 $\mu\text{g/ml}$ (green lines) and 400 $\mu\text{g/ml}$ (red lines) respectively. (Inset: Close up image of “ ΔD vs Time” plot at the point of nanoparticle solution injection)

Flow was continued for 4 minutes in order to fill the flow modules, and then adsorption under static conditions was monitored. Changes in frequency and dissipation of the silica crystals were recorded both during initial flow and static modes. ΔF of crystal 1 that was exposed to the lowest concentration (50 $\mu\text{g/ml}$) reached a constant value of -72 Hz in about 14.5 minutes, indicating no further adsorption of nanoparticles. Whereas frequency changes of crystals 2 and

3, exposed to higher concentrations (150 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$ respectively), took a longer time (~ 23 min) to reach their saturation values. Final ΔF s of crystal 2 and 3 at the saturation point in static mode were higher (~ 130 Hz) than that of crystal 1 (-72 Hz) indicating that the particles coverage on crystal 1 is significantly less than coverage on crystals 2 and 3 in the *static mode*.

Once the frequency and dissipation changes stabilized, flow of the corresponding nanoparticle solutions was resumed over the initially-adsorbed layers. This resulted in further significant decreases in ΔF of crystal 1 and relatively smaller decrease in ΔF s of crystals 2 and 3. These results indicate that the lower concentrations probably did not have enough nanoparticles accessible to the silica surface in *static mode* therefore resulting in only partial coverage (49% on crystal 1). Whereas higher concentrations had sufficient amount of nanoparticles to largely cover the silica surface (91% and 93% on crystal 2 and 3 respectively).

Further, continuous flow of the nanoparticle solution enabled additional nanoparticles to immobilize onto the crystal surface, leading to a fully saturated layer. It is interesting to note that the final value of ΔF change (Figure 3) is similar in all the three cases indicating that concentration of nanoparticles does not affect the surface coverage as long as the solution is in *flow mode*. In the *static mode* however, better coverage was obtained with higher concentrations.

Washing the adsorbed nanoparticles layer with DI water did not remove any significant mass which indicates that these particles are stably adsorbed on the silica surfaces and there are no detectable loosely bound nanoparticles present on the surface.

1.3 Conclusion:

The Au/Ag nanoparticles form very rigid layers on silica surfaces that can be defined by the Sauerbrey equation where frequency change is linearly proportional to adsorbed mass. Attachment of these nanoparticles to the silica surface is quite stable and not affected after several hours rinsing in DI water.

At lower concentrations, the particles adsorbed at a slower rate as compared to the higher concentrations. In *static mode*, lower concentration reached saturation much more rapidly than higher concentrations but with a much lower value of frequency change. This indicates a partial coverage of $\sim 50\%$ whereas the coverage at higher concentration was close to 92%. This result indicates that in *static mode*, better surface coverage is obtained with higher concentrations.

A coverage close to one hundred percent, was achieved in the flow mode of the nanoparticle solutions. The final

frequency change was the same in all three cases indicating that the concentration of the solution does not have any influence on the extent of surface coverage in *Flow mode*. However, rates of adsorption would be affected by concentrations.

2 OTHER EXAMPLES OF QCM-D APPLICATIONS

2.1 Phage Display – Titanium Specific Peptides³

Peptides that bind specifically to various metals and metal oxides have great potential in the nanotechnology/semiconductor industry to probe nano-scale structures.

Sano et al., have developed phage viruses with specific peptide sequences that specifically bind titanium surfaces. The peptides were isolated by several rounds of biopanning and their specificity towards titanium (Ti) was verified by QCM-D. In the biopanning process, a diverse ($\sim 2.7 \times 10^9$ sequences) phage display library was exposed to titanium oxide beads. The phages with affinity towards Ti bound to the Ti surface and the rest remained in solution. The non-bound phages were then removed from the solution and bound phases were separated from the beads. The phages specific for Ti were then magnified by infection of *E. coli*.

After several rounds of biopanning, when sufficient specificity was reached, the phages are separated and exposed to Ti crystal sensor mounted in a QCM-D chamber. As a control experiment, phage library of mixture of random DNA sequences was exposed to a control Ti surface. QCM-D results showed comparable shifts in ΔF in both control and experimental cases whereas a huge increase in ΔD was observed for adsorption of Ti specific phages. Change in ΔD for control phage was minimal. Significant increase in ΔD suggests that only very end of the ϕ Ti-12-3-1 phages interacted with the Ti surface and therefore take on a vertical orientation. This orientation promotes a greater amount of trapped water therefore increasing the viscoelastic properties of the film (high dissipation value). Control phages were randomly oriented on the Ti surface and were rigid (low dissipation value).

2.2 Build-up of polyelectrolyte multilayers⁴

Halthur et al., have reported in-situ characterization of a multilayer build-up of poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL) on silica and titanium surfaces, with and without an initial layer of polyethyleneimine (PEI), by means of ellipsometry and QCM-D.

A biphasic buildup was found in all systems, where the length of the first slow-growing phase is dependent on the structure of the initial layers. In the second, fast-growing

phase, the film thickness grows linearly while the mass increases in a nearly exponential fashion with the number of deposited layers. Combination of ELP (measuring dry mass) and QCM-D (measuring wet mass) enables calculation of water content that show that the film density increases in the later phase as the multilayer film builds up.

2.3 Characterization of DNA-PNA Hybridization⁵

Höök et al., have used QCM-D to characterize the bound state of single-stranded peptide nucleic acid (PNA) and deoxyribose nucleic acid (DNA) in relation to their ability to function as selective probes for fully complementary and single-mismatch DNA. Two different immobilization strategies of single-stranded PNA and DNA have been explored (thiol coupling to gold and biotin coupling to streptavidin).

The QCM-D technique has been used to characterize the successive steps of various immobilization strategies of single stranded PNA and DNA and the subsequent hybridization with fully complementary and various mismatched sequences of DNA. Both thiol-PNA and thiol-DNA bound to gold surfaces were rigid (low ΔD) and did not have affinity towards fully complementary DNA. Biotin-PNA and biotin-DNA coupled to a 2-D streptavidin layer, were bound in a flexible state (high ΔD), and had high ability to hybridize with both single mismatch and fully complementary DNA.

SUMMARY

QCM-D is a powerful tool for the study of nano-scale interactions and/or reactions on various surfaces. By measuring both changes in frequency and energy dissipation of the oscillating quartz, at various overtones, useful information regarding the viscoelastic properties of materials can be obtained in real time. High sensitivity of this technique allows in-situ detection of structural changes such as conformational changes, cross linking, swelling, etc. during and/or after the adsorption process.

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