

qCell T auto – a fully automated QCM-D platform for surface interaction analysis

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This study introduces the qCell T auto as a reliable and efficient tool for effortless long-time measurements that otherwise would have required the full hands-on attention of trained personnel. It carries all essential features of advanced Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) technology. In addition it is equipped with superior fluidic and sample control (Figure 1), electronic log filing, sensor serial numbers combined with an in-built data base paving its way towards becoming a standardized method. More specifically, 3T analytik monitored the gradual build-up of two types of polyelectrolyte multilayers (PEMs). The combined measurement of frequency and dissipation/damping provided important information on the viscoelastic properties of the various PEMs as well as the film thickness.



Fig. 1: The qCell T auto setup from 3T analytik featuring a dual sensor channel configuration with easy connection to potentiostat for combined electrochemistry and QCM-D measurements. Naturally, it also contains fully automated liquid, sample and temperature control.

What is QCM-D and what can it do?

Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) is a surface-sensitive technique that is applied to detect adsorption of a material to an oscillating quartz sensor. The adsorption process is detected by means of monitoring changes in the sensor's vibrational behaviour in real time. It can be implemented for studies of basically any surface adsorption phenomena including, but not limited to, molecular adsorption, bacterial adhesion or nanoparticle deposition, with new applications still being discovered. Negative shifts in the resonance frequency of the quartz sensor (Δf) is related to the amount of adsorbed mass with a sensitivity of about a billionth of a gram. The energy required to maintain constant vibrational amplitude is affected by dissipative energy losses induced by the adsorbed mass, also known as damping (half-bandwidth of the oscillation peak, $\Delta\Gamma$). Both parameters are utilized to characterize the adsorbed material, as schematically illustrated in Figure 2.

The simultaneous measurement of frequency and damping is an essential feature when QCM-D is performed in liquids, as without it, the liquid's influence on the mechanical

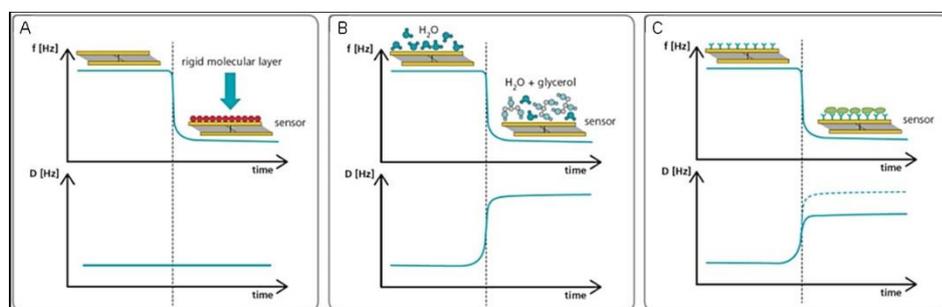


Fig. 2: Frequency and damping of three characteristic examples, (A) A thin, rigid layer (e.g. a thin protein layer or a thin metal layer) will cause a shift in frequency alone, without a change in damping. (B) A change of viscosity of purely Newtonian liquids (e.g. water, treacle, milk or fruit juice) will cause a decrease in frequency and an increase in damping, to equal amounts. (C) A viscoelastic layer (e.g. layers of polymers, biofilms, bacteria or cells) results likewise in a frequency and damping shift in opposite directions, but with different amplitudes.

characteristics of a sample cannot be accounted for. Homogeneous and predominantly elastic layers (i.e., rigid films, e.g., small, stiff and dehydrated molecules) dissipate little-to-no energy, resulting in a decrease in frequency but virtually no increase in damping (Figure 2A). For such layers, the so-called Sauerbrey relation [1] can be used to determine the adsorbed mass. Purely viscous layers (Newtonian liquids) dissipate much energy, resulting in a large decrease in

frequency and an equally large increase in damping (Figure 2B). The frequency is then governed by the liquid's viscosity as described by the Kanazawa equation [2]. Viscoelastic layers (e.g., large, 'soft' and hydrated molecules) possess both viscous and elastic characteristics thus dissipating an intermediate amount of energy. This results in a decrease in frequency and an increase in damping, albeit less in magnitude (Figure 2C).

In this situation, a viscoelastic module is applied to determine the adsorbed mass [3].

What are the advantages of 3T analytik automated QCM-D?

QCM, with or without Dissipation monitoring, has become a well-established tool for investigating surface associated phenomena. However, applying it on large scale routine basis requires efficient and simple means of developing and implementing standard operating procedures. To bring the technique to its full potential, simplifying its implementation on large scale routine basis, 3T analytik has taken a holistic approach to improve the QCM-D experience. To this end, 3T analytik has introduced simplified handling and operation, together with automated functions for experimental execution, high-standard log keeping, post-data processing and data management.

The auto sampling unit LiquiBox compact, included in the qCell T auto configuration, relieves the researcher from the headache of executing experiments identically. Parameters such as flow rates, sample selection, temperatures and triggering of external devices (e.g., potentiostat for combined QCM-D and electrochemistry measurements) are automatically controlled through the script function. This assures the identical execution of user-defined standard operating procedures (SOPs). Experimentation can thereby be fulfilled without the constant attention of trained personnel. Long term measurements can be supervised from distance through remote control. After measurements, data evaluation using the post processing qGraph Viewer software grants simple approach to, among other functions, align curves, calculate mass, thickness, nanoparticle sizes and kinetic constants. The processed data can be conveniently exported to Excel using an inbuilt macro function.

The patented sensor chip design ensures easy handling of the quartz crystal sensor and identical insertion of the sensor. Even more importantly, it is labelled with a sensor specific serial number to which each measurement is linked (Figure 3a). By labelling each sensor chip with a unique serial number, each sensor got its own identity, and this was the first step towards creating a robust data management system. The sensor specific serial numbers provides effortless means of keeping record of sensors. A referencing procedure and stability check at the beginning of a measurement ensures the quality of the sensor. Any further changes made, either inside or outside the measurement chamber, are being recognized by the instrument.

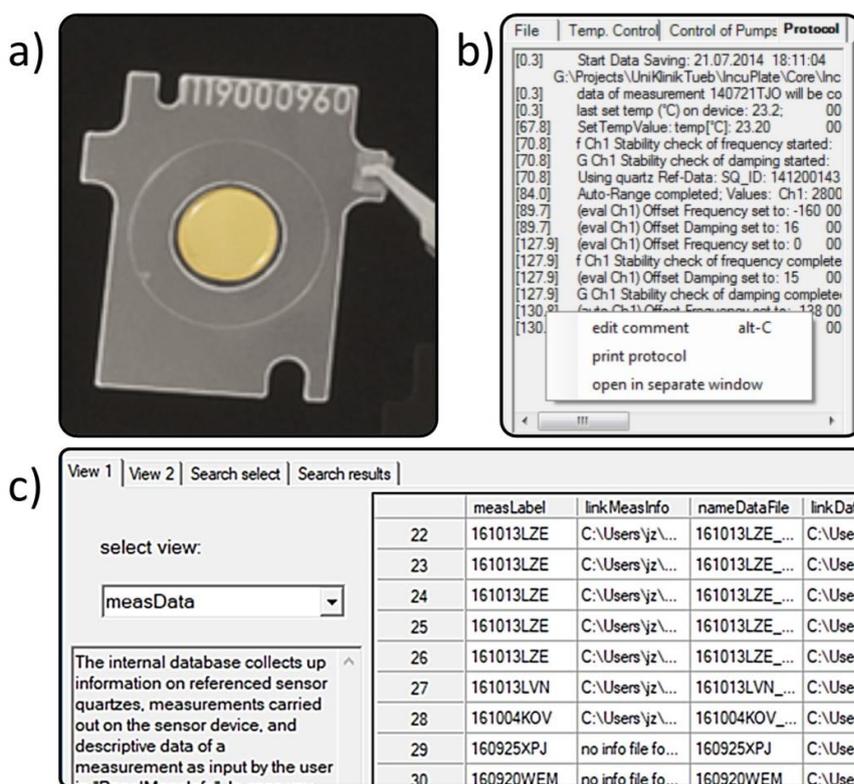


Fig. 3: 3T analytik has developed a robust quality control and data management system through implementation of
 a) serial number labelled sensor chips,
 b) electronic log file of all steps during measurements and
 c) easy access of data via an internal data base that automatically catalogues and organizes all measurements.

The data acquisition software qGraph keeps a detailed and time resolved log file of all steps during measurements in a standardised manner. The generated log file is automatically tied to the serial number printed on each sensor chip. The measurement file data is easily navigated using the log file (Figure 3b).

Furthermore, all measurements are automatically catalogued in an in-built database. There they can be sorted and accessed by a variety of conditions, including; sensor serial number, date of entry, operator, sample, substrate and other user-defined categories (Figure 3c). In other words, the detailed history of each sensor is by default carefully documented and conveniently organized. This simplifies any laboursome and time consuming 'detective work' for finding and analysing past data.

Experimental demonstration of the qCell T auto function capabilities

To demonstrate the power of the fully automated qCell T auto setup, 3T analytik performed experiments on the alternate deposition of poly-anions and poly-cations during the formation of polyelectrolyte multilayers (PEMs) [4], as schematically shown in Figure 4.

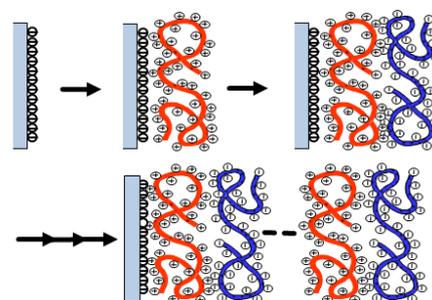


Fig. 4: Schematic representation of the layer-by-layer build-up of PEMs. After layering of a suitable surface with primer 'anchoring layer' (light blue), the multi-layers are assembled by up to 12 cycles of consecutive steps of rinsing, polycation addition (red), rinsing, polyanion addition (blue) and final rinsing.

PEMs have received considerable attention over the past decade due to their widespread potential applications that range from implant coating up to filtration devices or specialized optical coatings. The layer-by-layer process is very versatile and simple to apply on substrates of almost any shape but can be time consuming to perform, particularly when the film contains many layers and cycling between various polyelectrolytes is done manually.

Two kinds of PEM exist: those whose thickness grows linearly [5] with the number of deposition steps and those which grow exponentially [6]. Linearly growing films are generally dense, nicely stratified and impermeable to macromolecules. Exponentially growing films are, on the other hand, more gel-like, less structured and permeable to certain polyelectrolytes and proteins [7].

To further demonstrate the importance of dissipation monitoring for distinguishing between different types of films, we herein studied examples of both kinds of PEM; namely, the linear growth of polystyrene sulfonate/polyallylamine (PSS/PAH) and the exponential growth of poly-L-glutamic acid/polyallylamine (PGA/PAH). The polyelectrolyte solutions were prepared by dissolving the appropriate amounts of polyelectrolytes in Tris-NaCl buffer solution (Tris 20 mM, 150 mM NaCl, pH 7.4). As primer ('anchoring layer'), PEI (branched poly(ethylene imine) solution was prepared at 1 mg/mL and PAH, PGA, PSS at 0.5 mg/mL.

The in-situ monitoring of the PEM build-up process was carried out by the qCell T auto device according to a pre-scripted protocol programed to automatically alternate between the different polyelectrolytes. Subsequent PEM mass/thickness calculations were performed implementing the Sauerbrey relation and the viscoelastic module provided with the accompanying data processing software qGraph Viewer.

Results of PEM build-up in the 3T analytik qCell T auto

The frequency and damping progression during the build-up of PEI-PSS/PAH multilayers are shown in Figure 5A, top panel. A negative frequency shift corresponds to a mass increase. The damping value is related to the energy of dissipation of the crystal oscillation. A comparatively small and proportional increase of the damping value corresponds to the deposition of a rigid film (Figure 5A, top panel, blue line). Conspicuously, the build-up of the PEI-PGA/PAH multilayer is marked by a pronounced frequency shift following an exponential curve (Figure 5A, bottom panel, red line), indicative of the exponentially increasing mass deposition. At the same time, the damping value increases dramatically equally following an exponential curve (blue line), indicative of progressively viscous state of the PGA/PAH multilayer.

The PSS/PAH multilayer's thickness is determined to be approximately 50 nm after 10 cycles of PSS/PAH deposition by the Sauerbrey module [1] procedure (Figure 5B, top panel). Remarkably, in case of the

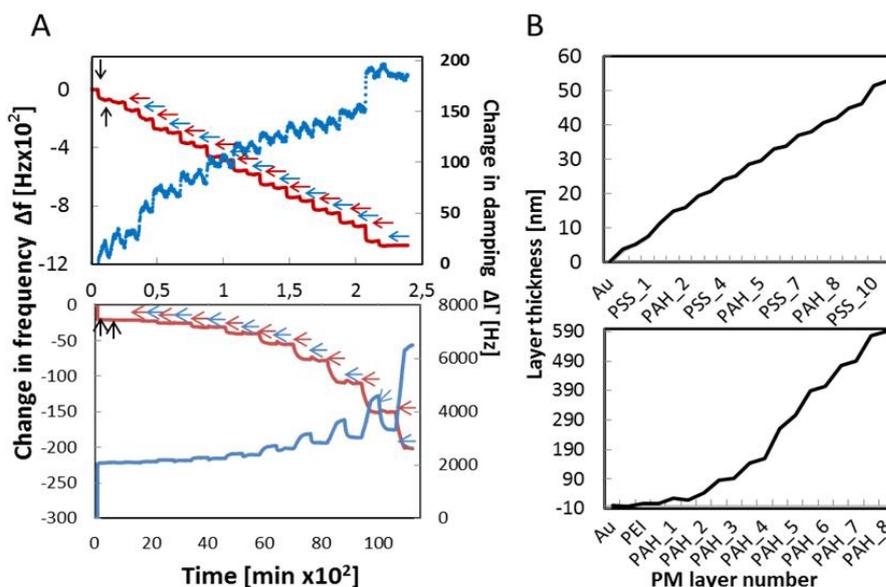


Fig. 5: Real time monitoring of the layer-by-layer build-up of PEI-(PSS/PAH) 10 and PEI-(PGA/PAH) 8 multilayers.

(A) Progression of the frequency (red line) and the damping (blue line) signals, measured by 3T analytik QCM-D, as a function of time. The arrows point to polyelectrolyte (PE) addition (PEI black, PSS/PGA red, PAH blue). Note, the 10 cycles build-up procedure for PSS/ PAH (top panel) and the 8 cycles build-up procedure for PGA/ PAH (bottom panel).

(B) The PSS/PAH and PGA/PAH film thickness as a function of PE addition. The PEM layer thickness has been determined by applying the Sauerbrey (top panel) and the viscoelastic module, (bottom panel) available in the accompanying software package. The film density was fixed at 1 g/cm³ for the Sauerbrey module and for the viscoelastic module, the layer density is fixed a 1 g/cm³, liquid density at 1 g/cm³ and the liquid viscosity at 1 mPa s. Note, the tenfold increase in layer thickness of the PGA/PAH PEM as compared to the PSS/ PAH PEM.

PGA/PAH multilayer, the film has a thickness of approximately 590 nm after eight cycles of PE depositions as calculated by the viscoelastic module [8] (Figure 5B, bottom panel). The dramatic increase in layer thickness as compared to the one of the PSS/PAH has been explained previously by a distinct mode of assembly [5]. Upon deposition of PGA, the PGA chains diffuse into the film forming a reservoir. When PAH is deposited, PGA chains diffuse out of the film and form a complex with the PAH chains forming a new layer. They are replaced by H₂O molecules, thereby increasing viscosity of the formed film. The layer thickness thus increases with the number of deposition steps due to the increase of the film thickness (the reservoir) resulting in the exponential growth of the PGA/PAH layer thickness. Overall, the characteristics of PSS/PAH and PGA/PAH depositions are much similar to previously published data on these PEMs [9 & 10].

Summary/Conclusions

The build-up of two different PEMs was successfully monitored in real-time by the 3T analytik QCM-D technology. Auto setup measurements enabled minimization of the hands-on time to approximately 5 min. The observed frequency shifts and damping progressions are clearly distinct for the two PEMs and point

to linear and exponential build of 10 and 8 layers of different physical states. The film thickness of the PSS/PAH and PGA/PAH multilayers on the sensor surface have been determined by the 3T analytik qGraph Viewer software to amount to ~50 nm and ~590 nm, respectively. As a final point, this study introduces the qCell T auto as a reliable and efficient tool for long-time measurements and enables experimental setups that would have been time and effort intensive to perform by manual sample handling. Auto setup measurements efficiently minimize of the hands-on time.

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