Sensors and Actuators B 214 (2015) 174–180

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Quartz crystal microbalance with microfluidic multi-stream solution control for mineralization kinetic analysis

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ARTICLE INFO

Article history:
Received 1 February 2015
Received in revised form 6 March 2015
Accepted 18 March 2015
Available online 25 March 2015

Keywords:
QCM
Microfluidics
Sensor
Microscopy
Mineralization
Calcium phosphate

ABSTRACT

We present a quartz crystal microbalance (QCM) sensor integrated with microfluidic multi-stream solution control for a variety of analysis applications, including improved studies of mineralization kinetics. The cost-effective assembly of this device and functionality of the QCM crystal in situations of rigid and viscoelastic mass loading are demonstrated. The significant advantage of this system is the control of solution mixing on-chip through the use of laminar parallel-flow which creates a liquid–liquid reaction interface at the QCM sensor. This shows improved sensor response times, due to laminar flow, of 105 Hz/min compared to 19 Hz/min for turbulent mixing in a bulk solution flow cell. This device adds to the current real-time mass measurements obtained from traditional QCM by allowing for simultaneous optical microscopy. The combination of QCM mass analysis, optical microscopy, and laminar parallel-flow to achieve a liquid–liquid reaction interface provides an analytical system well suited for the study of controlled mineralization. The microfluidic solution flow decreases non-specific mineralization and offers significant experimental control.

1. Introduction

Quartz crystal microbalance technology has been used over the past decades in many chemical, biological, and materials systems [1–8]. Recent work has focused on analysis of viscoelastic properties with QCM [3,9,10], development of multi-channel QCM [1,11], and integration of QCM with small-volume microfluidic systems. For example, miniaturized flow cells for QCM have been reported with 10–30 μL dead volumes [12,13] and micro-QCM analysis of airborne particles at the liquid–air interface has been achieved [14,15]. Small-volume QCM cassettes have been developed for multiplexing and automated protein analysis [11], digital microfluidics have allowed for QCM analysis of single droplet reactions [16], and sophisticated lab-on-chip microsystems have allowed for on-chip cell culture and QCM cell-profusion studies [17]. These examples demonstrate the successful QCM integration with microfluidic systems. One particular advantage of microfluidics is an ability to create laminar flow multi-stream parallel liquids where diffusion, mixing, and reaction at the liquid–liquid interface can be controlled by adjusting flow velocities [18–20]. Kamholz et al. demonstrated an example where protein binding to a fluorophore could be quantified using a T-sensor multi-stream microfluidic device [18]. Such carefully controlled mixing has not been previously integrated with QCM analysis but could offer particular advantages in a variety of applications, including the study of solid-forming reactions where uncontrolled or non-specific mass deposition would confuse QCM mass analysis.

QCM has previously been used to study solid-forming reactions [21–25], due to high sensitivity to rigid mass deposition, excellent time resolution for kinetic analysis, and convenient experimental set-up with gravimetric chemical reactions. Recent studies have used QCM to assess the effectiveness of templates in mineralization, including amelogenin and silicatein proteins [21,25], poly-aspartic acid and chitosan polymers [22,26], and DNA polynucleotides [24]. Commercial QCM systems are well-suited to these types of mineralization studies for the reasons described above, but lack control over solution mixing and preclude in situ optical analysis of mineral morphology (Fig. 1D). The lack of solution control has resulted in experiments proceeding with pre-mixed precursors, such as Ca^{2+} and PO_4^{3-}, which can result in homogenous mineralization in solution prior to entering the QCM flow cell. Ngourn et al. [24] implemented partial solution control by using in-line solution mixing with a y-junction immediately prior to entering the QCM cell. This limited non-specific, untemplated mineralization, but did not remove it entirely. In these cases, it is difficult or impossible to differentiate between heterogeneous mineralization at the surface/solution interface versus homogenous mineralization in solution followed by mineral particle adsorption to the QCM

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http://dx.doi.org/10.1016/j.snb.2015.03.030
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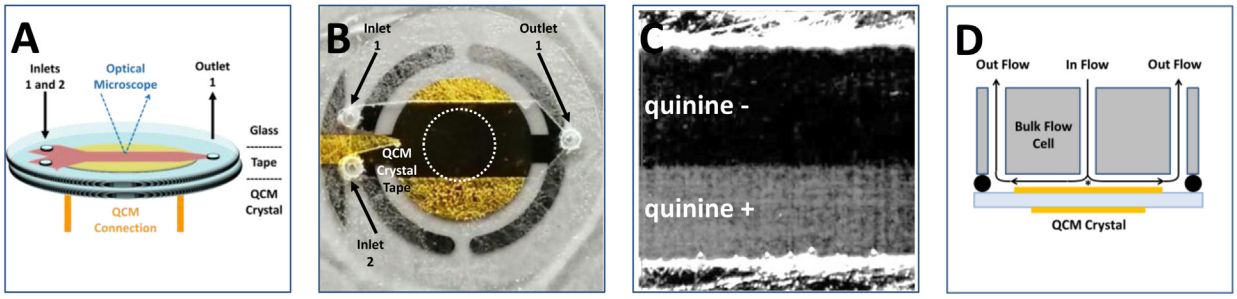

Fig. 1. Sensor design and fluidics with comparison to a turbulent bulk solution flow cell. (A) Diagram of microfluidic cell attached to commercial QCM quartz crystal and integrated with bright field optical microscopy. (B) Image of a 1 in QCM crystal with attached tape and glass, but no inlet/outlet tubing. This channel design allows laminar flow of two side-by-side liquids and leaves the sensitive area of the QCM (dashed circle) unencumbered. (C) Fluorescence microscopy of laminar flow when quinine is introduced at inlet 2, but not inlet 1. Laminar flow and limited mixing at the solution interface is observed. (D) Bulk solution flow cell diagram is shown for comparison. Turbulent mixing and a stagnation point (*) are observed when the single-stream solution approaches the QCM surface.

surface. Control over solution mixing at the QCM area of sensitivity would provide significant improvement in analysis. This can be achieved through the integration of microfluidic multi-stream laminar flow of two solutions on the QCM surface (Fig. 1). Experiments by Yin et al. [27] demonstrated the usefulness and elegance of mineralization studies in microfluidic devices at a liquid–liquid interface, but relied only on optical and spectroscopic analysis. Mineralization has also been studied in microfluidic tubular structures, but without solution mixing control [28]. Significant advances have been made in the integration of microfluidics with QCM, as discussed above, but none have achieved laminar flow solution mixing at the QCM surface.

The addition of laminar flow solution mixing to QCM would be beneficial in mineralization studies at the liquid–liquid–surface interface, but also for biosensor development in reducing reaction volumes, and in controlling homogenous chemical reactions immediately prior to surface functionalization reaction. Integration of an optical window for visualization of the QCM surface would also benefit the study of mineralization reactions, providing information on mineral morphology, and opening the door to integrative spectroscopic analysis, including micro-fluorescence or micro-Raman analysis.

This research demonstrates the design, assembly, and characterization of an integrated analytical sensor with a variety of possible applications, but with particular relevance to the kinetic study of solid-forming reactions. A commercial quartz crystal microbalance (QCM) instrument has been integrated with microfluidic solution control to achieve the directed and laminar flow of two parallel-flow solutions to create a reactive liquid–liquid interface at the QCM mass-sensitive surface. Optical microscopy has been included to monitor solid particle formation and morphology. The sensor allows for real-time, dynamic, and sensitive analysis and has a simple design, making assembly and use convenient. Dynamic sensor response to non-mass loading conditions, biomolecule mass loading, and rigid mineral mass loading are demonstrated.

2. Experimental

2.1. Reagents

Synthetic DNA strands were purchased from Integrated DNA Technologies with standard desalting and used after reconstitution in Tris buffer (5.5 mM trizma hydrochloride, 2.6 mM magnesium chloride, 10 mM sodium chloride, pH 7.3, filtered to 0.22 μm). Synthesis and purity were confirmed by the vendor using mass spectrometry. The following sequence was used: 5′-AGCAGCACAG AGGTCAGATG-3′. Molecules 6-mercapto-1-hexanol, 1-hexanethiol (95%), and bovine serum albumin (BSA, ≥98%) were purchased and used as received from Sigma–Aldrich. Quinine sulfate dihydrate (≥99.0%) was purchased from Fluka. All other molecules were reagent grade and were used as received.

2.2. Quartz crystal microbalance

Measurements were made using a commercial research quartz crystal microbalance (RQCM, Inficon) and 1 in. diameter Ti/Au polished 5 MHz quartz crystals (Stanford Research Systems). The RQCM provided frequency and loading resistance measurements. QCM crystals were cleaned between each experiment with 6 M hydrochloric acid, and then piranha (*Caution*: Handle piranha solutions with care), a 3/1 mixture of concentrated sulfuric acid and 30% hydrogen peroxide, and then rinsed with DI water and ethanol. A two-channel Masterflex C/L peristaltic pump with Tygon tubing (Cole-Parmer 0.020 in ID 0.092 OD) was used to deliver fluids at various flow rates for each experiment. Flow rate was calibrated using the mass of deionized water delivered over a period of time. Fresh tubing was used before each run to keep contamination to a minimum and limit flow rate deviations. When making large volume (bulk) solution measurements, a Kynar crystal holder and 100 μL flow cell (Inficon) were used with crystal face position 90° to ground (Fig. 1D). For microfluidic measurements, a CHC-15 (Inficon) crystal holder was adapted by mechanically removing plastic around the lip of the holder to accommodate a tight junction with the microfluidic cell and spring-loaded pogo electrodes were used to achieve a consistent electrical connection (Supporting Information, S1).

2.3. Microfluidic flow cell design, fabrication, and characterization

The microfluidic flow cell was built using a QCM crystal as the base or bottom layer. The microfluidic flow chamber was cut from Secure Seal doubled-sided 0.12 mm thick adhesive tape (Grace Bio-labs) using a desktop craft cutter, Silhouette Cameo Cutter (Silhouette) [29]. A flow chamber width of 7 mm was used and characterized. A double-width (75 mm × 50 mm) glass microscope slide (Ted Pella Inc.) was used at the top to seal the device and provide an optical window. Holes were drilled in the glass slide for fluid flow using a Dremel tool and 1 mm diamond drill bits. Tygon tubing (Cole Parmer) was attached to the glass slide using 5 min epoxy prior to assembly of the device. Alignment of the QCM crystal, adhesive tape, and glass slide was completed manually. Binder clips were used to apply pressure to the assembled device and all tape and glue was allowed to dry at room temperature for 24 h. Additional 5 min epoxy was added to the edge of the QCM crystal to make a seal with the adhesive tape and glass slide to prevent leaks. Laminar flow was observed by flowing DI water in one inlet and 50 μM quinine in the second inlet. A handheld UV lamp was