Sensors and Actuators B 188 (2013) 400–404

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Short communication

Sequential acoustic detection of atrazine herbicide and carbofuran insecticide using a single micro-structured gold quartz crystal microbalance

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ARTICLE INFO

Article history:
Received 28 November 2012
Received in revised form 9 July 2013
Accepted 11 July 2013
Available online 19 July 2013

Keywords:
Structured quartz crystal microbalance
TEM-grid
Carbofuran
Atrazine
Sequential acoustic detection

ABSTRACT

In this work, a cost-effective protocol to fabricate acoustic micro-immunosensors based on a quartz crystal microbalance (QCM) is reported. Specifically, the patterned quartz crystal is prepared via an electron beam evaporation of gold through a commercial transmission electron microscope (TEM) grid as a mask. Two different patterned zones on the crystal electrode surface were functionalized either with the monoclonal anti-carbofuran or with anti-atrazine IgG antibody by using thiol chemistry. Different concentrations of antigens (carbofuran or atrazine) were deposited onto their corresponding antibody modified zones monitoring in situ the specific interaction between antibody and its antigen from both resonant frequency and resistance changes versus time. Two sets of control experiments, including antigen interactions with non-specific antibody and simple antigen deposition onto thiolated-QCM quartz crystal were also investigated. It was found that the proposed methodology allows sequential specific detection of 4.5 μM carbofuran and 4.6 μM atrazine, respectively.

1. Introduction

The quartz crystal microbalance (QCM) is widely used as the transducer for the acoustic biosensor, mainly because of its excellent sensitive piezoelectric properties [1–4]. Thanks to the pioneering work of Sauerbrey to derive the relationship between resonant frequency and mass deposited on crystal surface [5], the QCM devices have found widespread applications in gas phase detection [6–8] and thin film characterization [9]. However, the Sauerbrey equation is only valid for the rigid, thin and homogenous film deposition on crystal, which hinders its applications on soft and solvated interfaces involved in liquid phase [10,11]. Fortunately, a new model for QCM operation in liquid was proposed by Kanazawa and Gordon. According to the published work [12], if only one side of crystal is exposing to liquids, the frequency shift (ΔF) due to the liquid deposition on crystal surface can be described as:

$$\Delta F = -\left(\frac{F_0^{3/2}}{\sqrt{\pi \mu_q \rho_q}}\right)\sqrt{\rho_L \eta_L}$$

where F_0, μ_q and ρ_q are the characteristic resonant frequency, shear modulus ($2.947 \times 10^{11}\ g\,cm^{-1}\,s^{-2}$) and density ($2.648\ g\,cm^{-3}$) of the quartz crystal, respectively, while the ρ_L and η_L are the density and viscosity of the bulk liquid.

In terms of experimental development, there are different ways to perform QCM measurements in liquid phase, either by using impedance analysis or QCM with dissipation and with oscillator circuits [13]. From all the QCM measurements, essential parameters such as surface viscosity, flexibility, interfacial trapped solvents, changes of proteins conformations, etc. can be deduced, which is especially important for the affinity biosensor applications in liquid phase [14]. As consequences, the QCM based acoustic biosensors have been widely used in various configurations for evaluation of DNA hybridization [15], detection of viruses [16], bacteria [17], proteins [18], hormones [19] and pesticides [20]. Interestingly, the most publications used the whole gold crystal area to detect only one analyte. Moreover, the idea of sequential acoustic detection of biomolecules using a unique structured QCM-crystal was not been reported.

Atrazine, a typical herbicide, has been intensively used in the corn agriculture, being one of the major sources for the environmental pollutions especially for the contamination of underground water systems [21]. Carbofuran is one of the highest toxic insecticides to human beings and the fatal dose to birds can be a single pesticide grain [22]. With these public concerns in mind, it is imperative to develop sensitive, fast and cost-effective analytical systems to specifically discriminate different bio-hazardous materials.

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http://dx.doi.org/10.1016/j.snb.2013.07.033

In the present work, a QCM quartz crystal was gold micro-structured with a help of one commercial TEM-grid via a well-controlled vacuum evaporation technique. The resulted micro-structured QCM crystal was further used as a platform for sensitive and specific acoustic immunosensing of different (bio)molecules. Thus, a successful immobilization of different antibodies (anti-atrazine IgG and anti-carbofuran IgG) onto different QCM patterns to sequentially detect two antigens (atrazine and carbofuran) is reported.

2. Experimental

2.1. Materials and instruments

11-Mercaptoundecanoic acid, N-(3-dimethylaminopropxyl)-N′-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide were purchased from Sigma–Aldrich (Schnelldorf, Germany). Atrazine, carbofuran, ethanol and acetone were acquired from Fluka (Lyon, France). Deionized water was produced by Millipore water purification system (Molsheim, France). Monoclonal anti-atrazine and anti-carbofuran IgG antibody produced in mouse (purified liquid) was obtained from antibodies-online GmbH (Aachen, Germany) while the monoclonal anti-human IgG antibody was purchased from Sigma (Lyon, France). The commercial transmission electron microscope (TEM) copper grids (Gilder Triple slot grid, 0.54 mm × 0.95 mm slots in a 3.05 mm copper grid) were obtained from TED Pella (US). Phosphate buffered saline (1× PBS, pH 7.4) was freshly prepared in our lab and used for preparation of antibody and antigen dilutions.

A QCM200 quartz crystal microbalance (Stanford Research System) including a crystal oscillator QCM25 and a polished AT-cut 5 MHz quartz with gold electrodes patterned on each side was used to record the resonant frequency. The gold evaporation is conducted by a vacuum metal evaporator (MEB 400, Plassys) using the electron beam mode. The optical microscope (Nikon, Eclipse LV100) and the field emission scanning electron microscope (Raith, FEG eLine) were used for the substrate surface characterization.

2.2. Acoustic immunosensor preparation

Firstly, the quartz crystal was washed in a mixed solvent solution of ethanol/acetone (10 mL, 1:1 (v/v)) for 12 h at room temperature. Secondly, a commercial TEM grid was fixed on the center of a gold crystal surface as a mask. Next, a gold evaporation process (thickness of 5 nm) was conducted through the grid mask, while the rest of quartz crystal surface was covered with a scotch tape to avoid the gold deposition over the whole crystal.

The resulted micro-patterned crystal was immersed into the 11-mercaptoundecanoic acid (MUA) ethanolic solution (1 mM, 10 mL) for 12 h at room temperature, followed by washing with ethanol and ddH_2O solutions. The resulted thiolated crystal was further activated with a mixed solution of EDC/NHS (0.2 mM/0.05 mM). After activation step ends, tiny amount of atrazine and carbofuran antibodies (0.6 μL, 0.1 mg/mL) were immobilized onto two different patterns of crystal at 4 °C over 5 h. The quartz crystal with antibody drops was placed in a sealed humid Petri-dish to avoid fast liquid evaporation. After washing and drying steps, the antibody-modified crystal was mounted in the QCM200 system and used for the specific and non-specific analyte acoustic investigations.

2.3. Acoustic immunosensor measurements

The QCM measurements were adapted from the simple micropipette-drop deposition procedure [20]. Specifically, 0.3 μL of different concentrations of antigen in PBS buffer (atrazine herbicide or carbofuran insecticide with concentration of 1 μg/mL, 1.5 μg/mL, 2 μg/mL, 5 μg/mL and 10 μg/mL) were successively deposited onto their corresponding antibody modified patterns.

For each antigen concentration, the incubation period onto the antibody modified patterned-surface was 10 min at room temperature, followed by the carefully local-washing with 1 μL ddH_2O for three times (between washing steps the QCM-system was fixed on “stand-by” to avoid any “false” frequency shifts). The system was shortly “turn-on” after the removed of the third water drop washing, monitoring the remained frequency base-line of a dried-crystal in air. At this stage the crystal was ready to be used for the next drop-deposition cycle of a given pollutant concentration. Further, the difference of resonant frequency of the dried quartz before and after antigen binding was calculated as the analytical signals to construct the calibration curves. Moreover, a pool mixture of atrazine and carbofuran (1:1 (v/v) volume ratio) was used for the acoustic measurements investigations on either the anti-IgG atrazine antibodies modified microspot to evaluate the QCM responses to atrazine or in the presence the anti IgG-carbofuran antibodies for its response to carbofuran compound.

Two sets of control experiments are proposed: one control investigated the QCM response of antigen binding to its non-specific antibody (atrazine to carbofuran IgG or carbofuran to anti-atrazine IgG), while the second control investigated the QCM response due to the direct antigen deposition onto a thiolated crystal without any specific atrazine/carbofuran-antibody modification.

The quartz crystal is regenerated after the atrazine and carbofuran detection measurements finished. Specifically, the regeneration is conducted by depositing 50 μL of 0.2 M NaOH solution onto the TEM grid patterned area for 30 min at room temperature, followed by ddH_2O washing, drying and another immobilization of two antibodies for 5 h at 4 °C onto different patterns, when the antibody modified crystal is ready for the second antigen QCM-measurement investigation. For one crystal, the regeneration step is repeated three times to evaluate the reusability of the QCM chip.

3. Results and discussion

The stepwise preparations of acoustic immunosensors are described in Fig. 1A, while the photos of TEM-modified gold QCM crystals and their SEM images are shown in Fig. 1B.

In the present work, a thin layer of gold (5 nm) was evaporated onto the crystal surface through a TEM-grid mask and consequently, a well-organized marker containing three independent slots was obtained. After carefully grid removing, the size of one pattern was measured as 0.6 mm × 1 mm which is very close to the grid pattern. Moreover, the thin deposed gold film has negligible influences onto the surface morphology of commercial QCM-crystal gold electrode, as indicated in the SEM images in Fig. S1 (supplementary data). The reproducibility of the drop-deposition measurements is highly improved, as individual drops can be precisely deposited onto the same position due to the presence of a visible TEM-grid marker on the crystal surface.

Since there are three gold TEM-patterns on the surface of a QCM-crystal, their frequency responses due to the successively deposition of 0.3 μL water drops onto each pattern were monitored (Fig. S2). It was found that the frequency response for the first (zone 1) and the third (zone 3) patterns are identical (Fig. 1B-SEM image). Further, these two micro-patterns were biofunctionalized with two different types of antibodies. The middle pattern was not modified with any antibodies and used to separate the two target sideways patterns in the presented acoustic experiments. Moreover, the resonant frequency shifts (Fig. 2) upon deposition of different concentrations of atrazine and carbofuran antigens were