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User-friendly, miniature biosensor flow cell for fragile high fundamental frequency quartz crystal resonators

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ABSTRACT

For the application of high fundamental frequency (HFF) quartz crystal resonators as ultra sensitive acoustic biosensors, a tailor-made quartz crystal microbalance (QCM) flow cell has been fabricated and tested. The cell permits an equally fast and easy installation and replacement of small and fragile HFF sensors. Usability and simple fabrication are two central features of the HFF-QCM flow cell. Mechanical, thermal, electrical and chemical requirements are considered. The design of the cell combines these, partially contradictory, requirements within a simple device. Central design concepts are discussed and a brief description of the fabrication, with a special focus on the preparation of crucial parts, is provided. For test measurements, the cell was equipped with a standard 50 MHz HFF resonator which had been surface-functionalised with a self-assembled monolayer of 1-octadecanethiol. The reliable performance is demonstrated with two types of experiments: the real time monitoring of phospholipid monolayer formation and its removal with detergent, as well as step-wise growth of a protein multilayer system by an alternating immobilisation of streptavidin and biotinylated immunoglobulin G.

1. Introduction

During the last decade acoustic biosensors which detect mass- and viscosity-alterations of surface adsorbed biofilms via a corresponding shift in resonance frequency (Steinem and Janshoff, 2007; Bizet et al., 1999; Ballantine et al., 1997), have found widespread acceptance as versatile tools for the analysis of biomolecular interactions and related phenomena. Especially the quartz crystal microbalance (QCM) with a thickness-shear mode (TSM) resonator in combination with a flow cell is gaining increasing relevance for biological and biochemical research (Steinem and Janshoff, 2007). This trend is well reflected by the steady growth of related publications as recently reported by Cooper and Singleton (2007), who documented, for the period 2001–2005, more than 1400 articles referencing "quartz crystal microbalance" or "QCM" in the Web of Science database.

Various properties of QCM systems may account for their extensive usage. Beside the scientific merits of QCM biosensors (Marx, 2007), the principle of operation is simple and the basic elements of such a system are easily available at relatively low costs. Hence, for many research groups home made systems with a reasonable performance are easy to build and an alternative to more sophisticated, but also far more expensive commercial machines.

A crucial factor for the performance of QCM systems – home made or commercial – is the fundamental resonance frequency, f_0, of the oscillating piezoelectric sensor. A mass alteration per unit area, Δm, of a thin biofilm on the sensor surface is, to a first order approximation, the main detectable effect of biochemical binding interactions under study. It can be measured via a corresponding frequency shift, Δf, according to the Sauerbrey relation (Sauerbrey, 1959) $\Delta f = -2f_0^2/\sqrt{\rho\mu} \cdot \Delta m$, with the density $\rho = 2648\,\text{g/cm}^3$, and the shear modulus $\mu = 29.47\,\text{GPa}$ (AT-cut) of the quartz crystal. Since the theoretical sensitivity increases with f_0^2, the use of a sensor with a high fundamental resonance frequency is clearly desirable. Following the resonance condition $f_0 = \sqrt{\mu/\rho}/2h$, the fabrication of TSM resonators with higher values for f_0 is basically achieved by reducing the plate thickness h of the sensor.

For practical purposes however, the minimum thickness is constrained by mechanical stability issues. Most applications utilise either 5 MHz or 10 MHz resonators, with a corresponding plate thickness of 0.33 mm or 0.17 mm. Such thicknesses, in combination with a diameter of approximately 14 mm, provide a sufficient mechanical stability for an easy installation in a flow cell. Occasionally sensors with resonance frequencies between 20 MHz and 30 MHz are used (Okahata et al., 2000, 2007; Sota et al., 2002; Michalzik et al., 2005; Sota et al., 2002). These sensors are smaller in diameter (approximately 8 mm) and already rather thin and fragile. The corresponding plate thicknesses between 83 μm and 56 μm

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require more careful handling, and also stress-free mounting to flow cells becomes increasingly difficult (Sota et al., 2002). Thus, a further improvement towards higher sensitivities may not be expected from the exploitation of even thinner standard resonators, though they are available up to about 45 MHz.

An alternate approach to higher frequencies is the utilisation of quartz resonators with an "inverted mesa" structure. These quartz disks with a diameter and thickness of about 5 mm and 0.1 mm, respectively, have a membrane of reduced thickness only in their small central circular area of about 5 mm^2. The surrounding thicker material provides a better mechanical stability and the small membrane can become as thin as 8.3 μm, resulting in a high fundamental frequency (HFF) up to $f_0 = 200$ MHz.

The first application of a 30 MHz HFF-QCM sensor in a liquid environment was demonstrated in 1993 by Lin et al. (1993) who used glucose and electrodeposited copper on the sensor surface to investigate the acoustic properties. The first application as a biosensor was presented eight years later. In a comparative study, Uttenthaler et al. (2001) investigated the detection of M13 phages in liquids and the acoustic properties of glycerol/water mixtures with four different HFF quartz crystals, operating at 39 MHz, 56 MHz, 70 MHz and 110 MHz, and with a 19 MHz standard quartz resonator. With respect to the 19 MHz standard the increase of the relative sensitivity with increasing frequency was even stronger than theoretically predicted. The 56 MHz sensor showed the best performance, with a relative improvement of the signal-to-noise ratio by a factor of 6.5 and an enhanced detection limit for phages by a factor of 200.

In spite of these encouraging results, it seems that HFF-QCM systems have still not yet appeared in research laboratories. The reason for this is rather obvious. High fundamental frequency quartz crystals are too small and, although mechanically more stable than standard resonators of equal frequency, they are still too fragile, which makes them difficult to handle and to support. Both, the small size and the fragile nature of a HFF resonator hardly allow the application of common design concepts which are frequently used for standard QCM flow cells. O-rings and electric spring contacts, as they find wide application in 5 MHz and 10 MHz systems, are not easy to down-scale. Standardised O-rings are much too hard and too bulky, electric spring contacts will find very little room to connect to the sensor and they would be tricky to fabricate. In addition, both elements will create a highly nonuniform stress distribution through the small quartz crystal and the active membrane unavoidably will be affected. It has been shown by Sota et al. (2002) that O-ring based holders for a 27 MHz quartz crystal resonator already cause less stable sensor signals. Therefore, most researchers who investigate and/or apply high frequency TSM biosensors glue the sensor irreversibly onto a larger carrier chip, e.g. see (Uttenthaler et al., 2001; Sota et al., 2002; Michalzik et al., 2005). This solution to the mounting problem actually works well, but it is very delicate, time consuming and awkward in practice. All HFF QCM sensors used in the study of Uttenthaler et al. (2001), for instance, were fabricated with bond wired crystals glued by a silicone adhesive to a carrier with open flow channels. In such a configuration the sensor crystal, once mounted, can hardly be removed from the carrier, a fact that might easily turn out to be annoying, if not problematic.

In the present work we introduce a revised version of a HFF-QCM flow cell which we have tailored to the special requirements of small and fragile HFF quartz resonators. The design allows for fast and reliable installation and replacement of a HFF sensor within a few seconds. This high user friendliness is a key feature of our system and it is accomplished without the use of complicated engineering or delicate parts. Both, the essential design guidelines and the manufacturing of crucial elements are discussed. We demonstrate the effective operation of the system with two kinds of experiments, both using a self-assembled monolayer (SAM) of 1-octadecanethiol on the gold-covered surface of a 50 MHz HFF resonator: In a first experiment we have tested the reversible functionalisation of an installed sensor by phospholipid monolayer formation from an aqueous buffer suspension of phospholipid vesicles, followed by complete lipid removal by the detergent octyl- β-D-glucopyranoside. In the second experiment, formation of an alternating protein multilayer composed of streptavidin and biotinylated immunoglobulin G was monitored.

2. Experimental

2.1. Requirements and design guidelines

The general purpose of a flow cell for QCM applications is primarily to support and connect the sensor mechanically and electrically in such a way that a flowing liquid can wet the sensitive surface in a defined region without leaking into the surrounding area. Whereas these demands are relatively easy to meet with large and stable quartz crystals, this becomes considerably more challenging for small and fragile HFF quartz resonators. For a user-friendly HFF-QCM flow cell however, there are even more requirements to specify: First, (i) the cell has to allow for fast, easy, and reliable installation and replacement of the HFF quartz sensor. Gluing or pre-wiring of the sensor shall be avoided by an effective clamping mechanism. (ii) The clamping mechanism has to act on the fragile quartz crystal with a gentle and uniform pressure; a shear-stress across the crystal must not build up. (iii) Under no circumstances, liquid must leak through the flow cell/sensor interface. Further, (iv) all materials which get in contact with the liquid have to be biologically inert. (v) All electrical lines and contacts shall be fit for high frequency signals; wires shall be short and with few discontinuities in impedance. (vi) The complete system shall be fully reusable with only little need for maintenance. Also important for a good performance are (vii) the thermal properties of the cell. A small heat capacity, in combination with a high heat conductivity, allows for fast and accurate temperature stabilisation via an external active control unit. Thermal fluctuations on the outer cell-surface shall not reach the flowing liquid, the sensor, and the probe volume. Thus, (viii) the probe volume itself shall be located in the centre, and the probe volume shall be very small, providing for fast sensor response. In addition to all these requirements, (ix) the fabrication should be inexpensive and technologically feasible even for a small workshop with conventional machine tools.

2.2. The flow cell, fabrication and assembling

All requirements mentioned above, and the related conflicting requirements of the constructions could be met and resolved with the design given in Fig. 1. For the purpose of a better illustration, the presented schematic outline is not drawn to scale and some features of small size are depicted out of proportion. A detailed exploded assembly drawing together with a complete part list and additional photographs are presented as supporting online material in Figs. S1 and S2 respectively.

The complete flow cell system measures 30 mm in diameter and 26 mm in height. The flow cell is made up of two main components: The lower flow-channel unit, which supports the HFF quartz crystal, constitutes the major part of the system. The top structure – the flow cell head – provides the high frequency signal via a planar electrode and simultaneously generates a uniform pressure upon the quartz crystal. The accurate positioning between the two parts is ensured by two precise guide bolts with threads which are tightly fixed to the lower unit and which stick through, and out of, the flow cell head, so that the two parts can be screwed together with two curled nuts on top. The quartz crystal is sandwiched in between these two parts.