IMPROVED PROTEIN BINDING RATE FOR BIO-SENSORS USING AC ELECTROOSMOSIS

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ABSTRACT

Many biosensors are limited by long detection times that result from transport limitations of large macromolecules such as proteins which must reach the active surface in order to be detected. To overcome this difficulty, a common biosensor, the quartz crystal microbalance was modified with a microelectrode pattern capable of producing AC electroosmosis. The mixing pattern that is thereby generated enhances mass transport to the sensor surface. A hypothetical detection threshold of 200 Hz was reached in 4.5 minutes with mixing compared to 71.8 minutes under static conditions thus showing an order of magnitude improvement in detection time.

KEYWORDS: Quartz Crystal Microbalance, AC Electrokinetics, Biosensor, Mixing

INTRODUCTION

In order for a biosensor to detect an analyte, target molecules must actually bind to the surface of the transducer. Since proteins often have very small diffusion coefficients (Hemoglobin - 6.9×10^{-7} cm^2/s, Immunoglobulin 4×10^{-7} cm^2/s) the binding of protein to a surface tends to be highly transport rate limited and can take several hours to reach equilibrium or exceed a detection threshold.[1] The slow response times of these sensors often precludes their use in handheld total analysis systems for rapid diagnostics. In order to overcome this difficulty, a common biosensor, the quartz crystal microbalance (QCM) was modified with a microelectrode pattern capable of producing AC Electrokinetc phenomena – AC electroosmosis (ACEO) in particular.[2]

THEORY

ACEO produced from interdigitated microelectrodes can cause rotational fluid velocity patterns (fig. 1) of sufficient velocity (up to hundreds of microns per second) to aid in the transport of fresh reagents to the binding surface of the sensor.[3] Initial results indicate greater than an order of magnitude reduction in detection time.

The QCM is a piezoelectric bulk acoustic wave sensor which consists of a thin wafer of AT-cut quartz sandwiched between two electrodes (usually gold) (fig. 2(a)). The resonant frequency of the QCM decreases as mass (such as protein) becomes attached to the surface. In biosensing applications, one electrode is placed in solution, functionalized and acts as the detection surface.

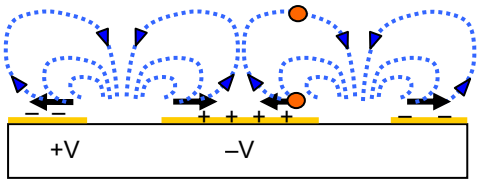

Figure 1: ACEO velocity pattern caused by interdigitated electrodes.

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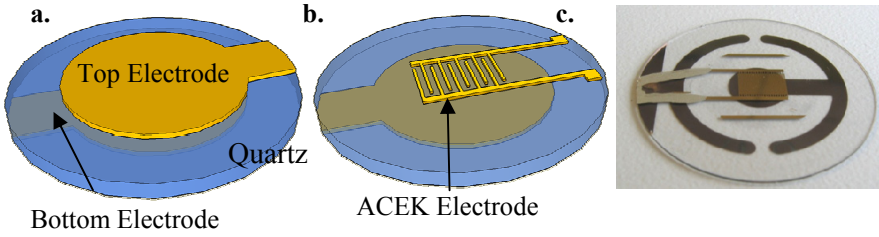

Figure 2: (a) Standard QCM, consisting of quartz sandwiched between two gold electrodes. (b) Modified QCM. The top electrode has been converted into a pair of interdigitated electrodes capable of generating ACEO. (c) Device Image

EXPERIMENTAL

In this paper, the sensing electrode has been replaced with a set of interdigitated electrodes using standard photolithography and wet etch procedures (fig. 2(b & c)). The interdigitated electrodes were 20 µm wide with a 20 µm gap. Modification of 5 MHz QCMs (SRS Inc.) in such a manner results in increased attenuation at resonant frequency (from ~ -0.5 dB to -8 dB) but an increase in the Q factor (~9,000 to ~11,000 in air). The modified device was placed in a flow chamber and attached to an HP 4395A network analyzer for measurements and a Wavetek 182A 4 MHz Function Generator, to apply a 1 kHz signal at 10 V (which generates a maximum field strength of 5E5 V/m) to the ACEO electrodes (fig. 3). Monitoring of the resonant frequency was carried out with Labview (National Instruments) software developed in-house. 20µL of 0.1 mg/mL goat IgG in DI water (0.0002 S/m) was injected into the chamber and allowed to incubate either under static conditions or with the use of ACEO mixing.

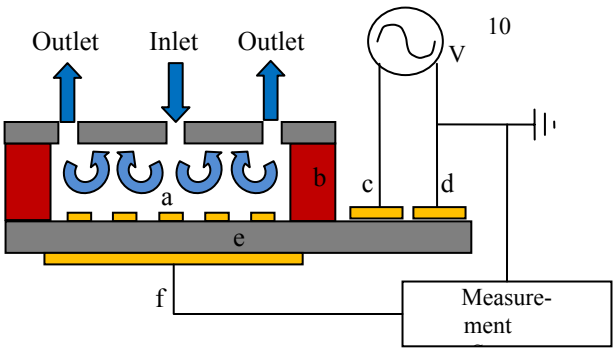

Figure 3: Experimental setup. (a) ACEO Electrodes and chamber interior, (b) chamber wall, (c) Left ACEO electrode, (d) Right ACEO electrode (see fig 2b), (e) quartz wafer, (f) QCM bottom electrode.

RESULTS AND DISCUSSION

The results, shown in figure 4, demonstrate the dramatic improvement that results from using the electrohydrodynamic mixing. A hypothetical detection threshold of 200 Hz was reached in 4.5 minutes with mixing compared to 71.8 minutes under static conditions thus showing an order of magnitude improvement in detection time. This corresponds to about 1.4 µg of IgG adsorbed according to the Sauerbrey equation.[4] Unexpectedly, not only was the detection time faster, but the amount of deposited mass was increased also. Equilibrium was not reached for several hours.

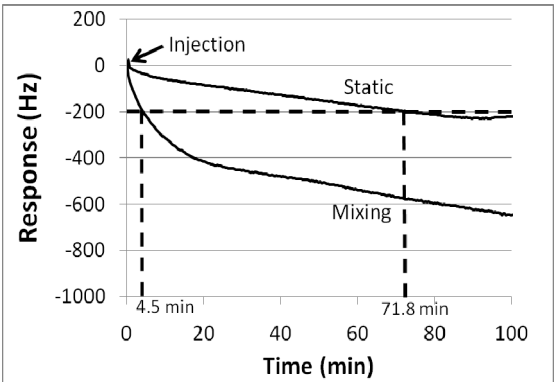

Figure 4: Sensor response to binding of antibody. A hypothetical detection threshold is shown at 200 Hz. Respective times to reach this threshold are shown on the x-axis.

Typically, a full detection procedure would consist of surface functionalization with a biologically selective layer followed by the introduction of a sample containing the analyte to be measured. Each step would cause a similar sensor response.

CONCLUSIONS

A similar increase in binding rate will likely be seen for many other biosensors equipped with such microfabricated electrodes and should not be limited to the QCM. Furthermore, the modified sensors can utilize a variety of AC electrokinetic phenomena, not just AC Electroosmosis. Electrothermal effect may be used in high conductivity solutions, such as biological media and dielectrophoresis may be used for larger particles such as viruses and bacteria. The improvement in binding rate demonstrated here can help realize the goal of rapid detection of biological samples in clinical, counter bioterrorism and food safety applications.

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