Application Note

QCMD immunosensor for small molecule analytes

Summary

A QCMD-based immunoassay for label-free analysis of small molecule concentration in industrial samples was developed using an AWS QCMD system with surface-modified 5 MHz fundamental frequency QCMD sensors. Accuracy and precision of the immunoassay is evaluated with respect to the industry-standard HPLC reference.

Introduction

Accurate, rapid, and cost-effective quantification of small-molecule analytes is a pressing problem in various industrial (food, agriculture, environmental protection) and health-related fields. Existing approaches include enzyme-linked immunosorbent assays (ELISAs) and various types of chromatography (e.g., High Performance Liquid Chromatography, HPLC). These approaches require trained personal and centralized laboratories, and their deployment in the field is difficult or impossible.

Quartz Crystal Microbalance with Dissipation, or QCMD, is emerging as a promising technology for the development of fast, portable, automated, and cost-effective immunosensors. Here, we demonstrate a competitive small molecule immunoassay based on the AWSensors QCMD technology.[1,2]

Materials and Methods

Materials. The experiments were performed in a three-channel AWSensors A20 Research Platform with a three-channel F20 Flow Platform for semi-automatic sample injection and flow handling. 5 MHz, 14 mm, gold-coated QCMD sensors and the respective flow cells. Changes in the resonance frequency and dissipation were recorded at 35 MHz (7th overtone) in three sensors at a time, mounted in the three channels. This procedure ensured that each sample or standard was analyzed in triplicate. Furthermore, on each sensor, each sample was run three times, resulting in nine independent determinations per sample.

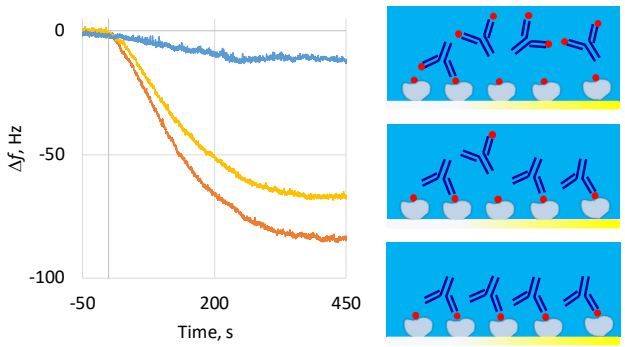

Figure 1: QCMD-based competitive immunoassay.

The plot on the left shows the changes in frequency due to antibody binding to the surface-immobilized analyte for three different analyte concentrations in solution, while the images on the right schematically depict what is happening in the assay. The analyte is represented with red circles and the antibody in blue. The analyte is coupled to a molecule of albumin (light grey) for immobilization.

The platforms and the sensors were cleaned according to the AWSensors cleaning protocols.[3] All experiments were performed at a constant temperature of 28°C ± 0.05°C.

Sensor functionalization. Gold-coated sensors were functionalized with oligo-ethylene glycol thiols,[4] a fraction of which had terminal carboxylic acid groups for immobilizing albumin-bearing analyte molecules following a standard immobilization protocol.[5,6,7] In some

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experiments, albumin-bearing analyte molecules were adsorbed on the surface of the sensors directly.

QCMD measurements. Functionalized sensors were mounted in the flow cells and installed in the research platform. PBS buffer was passed over the sensor surface at a flow rate of 25 µl/min. Once a sufficiently stable baseline was acquired, samples of analyte of known concentration (standards) in PBS buffer were injected into the flow using the injection capability of the F20 platform. Sensor surfaces were regenerated after each such injection by treating them with 0.1 M HCl, and the measurement was repeated for the next standard, or sample with an unknown analyte concentration. Standard measurements were used to construct calibration curves that were then used to determine the concentration of the analyte in the unknown samples. Sensors could be used for ~ 50 regeneration cycles over a period of 12 – 15 days, as long as they were maintained under continuous flow.

Analyte concentrations were analyzed in parallel with HPLC to provide reference values.

Results

Immunoassay format. The competitive QCMD-based immunoassay works as follows. The small molecule analyte, conjugated to a molecule of albumin, is immobilized on the surface, either directly, or via an oligoethylene glycol linker. In the absence of the analyte in solution, the antibody will bind to the analyte on the surface and induce a frequency shift, Δf_0. As the concentration of the analyte in solution increases, the amount of antibody binding to the surface-immobilized analyte will decrease, and eventually become negligible, and so will the frequency shift, Δf, measured with the QCMD. This is schematically illustrated in Figure 1. A ratio between the frequency shift at a given analyte concentration, Δf_i, and the frequency shift in the absence of the analyte, Δf_0, is taken and used in further analysis.

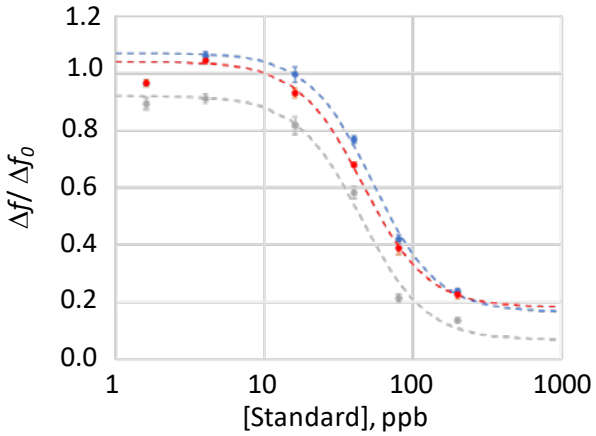

Figure 2: Calibration curves for three different sensors S1 (blue), S2 (red) and S3 (grey). Note that the analyte concentration is plotted in a logarithmic scale.

Sensor Calibration. The Δf_i / Δf_0 ratios for six standard analyte solutions are plotted against the analyte concentration in Figure 2. Calibration curves were fitted with a 4-parameter logistic equation $\frac{\Delta f}{\Delta f_0} = d + \frac{a-d}{1+\left(\frac{[Analyte]}{c}\right)^b}$, where *a*, *b*, *c*, and *d*, are the maximum signal, the Hill coefficient, the IC50, and the minimum (background) signal, respectively.[8]

	A	B	IC50 (PPB)	D
S1	1.05	2.4	52	0.20
S2	1.05	1.9	46	0.17
S3	0.88	3.1	45	0.12

Table 1: Sensor calibration parameters corresponding to Figure 2.

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Immunoassay Performance. Calibrated sensors were then used to determine analyte concentrations in real samples with unknown analyte concentrations. To evaluate the performance of the QCMD-based immunoassay, analyte concentrations were simultaneously determined in the same samples by HPLC. Because the sensitivity of HPLC is in ppm range, while the sensitivity of the immunoassay is in the ppb range, different dilutions had to be used for the two techniques.

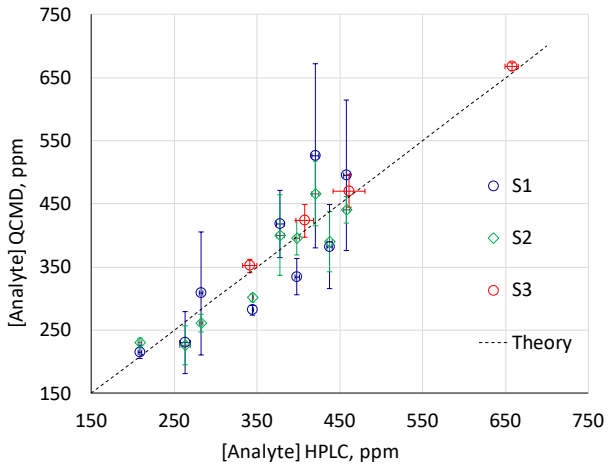

Figure 3: Comparison of analyte concentrations determined by QCM and the ones of HPLC.

The results are plotted in Figure 3 for three different sensors. For each sensor, each data point is an average of three independent determinations, and error bars are standard deviations. A dashed line depicts the expected correlation between the analyte concentrations determined by QCMD and HPLC.

A statistical measure of assay accuracy was calculated as root-mean-squared deviation between the analyte concentrations determined by QCMD and HPLC: $\boldsymbol{Accuracy} = \sum \sqrt{\frac{(\boldsymbol{Analyte_{HPLC} - Analyte_{QCMD}})^2}{\boldsymbol{N}}}$, where *N* is the number of measurements. For the three sensors shown in Figure 3, the values of accuracy were 15 ppm, 32 ppm and 55 ppm, with an average value over the three sensors of 40 ppm, while the precision was 30 ± 11 ppm, with *N* = 279 measurements.

Conclusions

We demonstrate how AWSensors QCMD technology can be used to design and implement a competitive immunoassay for a small-molecule analyte and evaluate its performance against industry-standard HPLC. Direct comparison of the two techniques performed on the same samples indicates that QCMD immunoassay can determine analyte concentration accurately and precisely, while reaching sensitivities in the ppb range at a significantly reduced cost.

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Order Information

Product	Quantity	Reference
AWS A20 RP - 3 channels	1	AWS A20 000013 A
AWS F20 FP - 3 channels	1	AWS F20 000023 A
QCM flow cell for AWS Equipment	3	AWS CLS 000021 Q
AWS QCM Au/Cr wrapped sensor, 5 MHz	10	AWS SNS 000042 A

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References

[1] A. Grammoustianou, E. Gizeli 2018, *Acoustic Wave–Based Immunoassays* in *Handbook of Immunoassay Technologies*, ed. Sandeep K. Vashist, John H.T. Luong, Academic Press, 203-239.

[2] AWSensors QCMD Technology Note can be found on the web at www.awsensors.com

[3] Available to AWSensors customers.

[4] J. Christopher Love, Lara A. Estroff, Jennah K. Kriebel, Ralph G. Nuzzo, George M. Whitesides *Self-Assembled Monolayers of Thiolates on Metals as a Form of Nanotechnology*. Chem. Rev. 2005, 105, 4, 1103-1170.

[5] N. Patel, M.C. Davies, M. Hartshorne, R.J. Heaton, C.J. Roberts, S.J.B. Tendler, P.M. Williams, Immobilization of protein molecules onto homogeneous and mixed carboxylate-terminated self-assembled monolayers, Langmuir. 13 (1997) 6485–6490. doi:10.1021/la970933h.

[6] R.E. Ducker, M.T. Montague, G.J. Leggett, A comparative investigation of methods for protein immobilization on self-assembled monolayers using glutaraldehyde, carbodiimide, and anhydride reagents, Biointerphases. 3 (2008) 59–65. doi:10.1116/1.2976451.

[7] March, C., Manclús, J. J., Jiménez, Y., Arnau, A., & Montoya, A. (2009). *A piezoelectric immunosensor for the determination of pesticide residues and metabolites in fruit juices*. Talanta, 78(3), 827–833. doi:10.1016/j.talanta.2008.12.058

[8] Findlay J.W., Dillard R.F. *Appropriate calibration curve fitting in ligand binding assays*. AAPS J. 2007, 9(2). E260-7.