DETECTION OF AFLATOXIN USING QUARTZ CRYSTAL MICROBALANCE

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Abstract. *Aflatoxins are naturally occurring mycotoxins that are produced by many species of Aspergillus, a fungus, most notably Aspergillus flavus and Aspergillus parasiticus. Aflatoxins are toxic and among the most carcinogenic substances known. They aren't destroyed by thermal procedures. Quartz Crystal Microbalance is an ultrasensitive piezoelectric method for detection of aflatoxin in concentrations under the value 4 ng/ml (ppb) – current maximum permitted levels set by European Commission. In this method, the quartz crystal surface was coated with a self-assembled monolayer of ethanethiol. Aflatoxins B1 and M1 were diluted in methanol. To obtain the calibration curve, several samples with various concentration of aflatoxin were measured.*

Keywords: *QCM, aflatoxin B1, self-assembled monolayer, frequency response.*

1. INTRODUCTION

Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, an inferior fungus, most notably being *Aspergillus flavus* (this give the name: A. Fla toxin) and *Aspergillus parasiticus*. Aflatoxins are toxic and among the most carcinogenic substances known [1]. After entering in the body, aflatoxins may be metabolized by the liver and become the less harmful aflatoxin M1.

It is very well known that the least thirteen different types of aflatoxin are produced in nature. Aflatoxin B1 (Fig. 2) is considered the most toxic and is produced by both *Aspergillus flavus* (Fig.1.) and *Aspergillus parasiticus*.

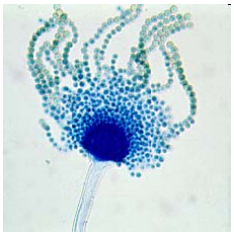

Fig. 1. Aspergillus flavus.

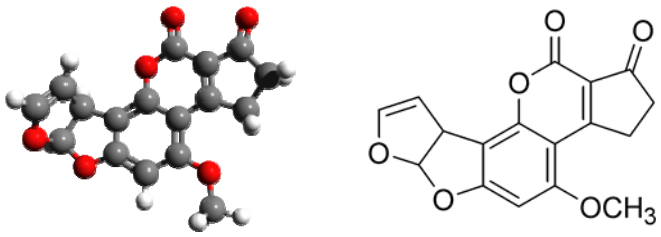

Fig. 2. Structure of aflatoxin B1.

Aflatoxin G1 and G2 are produced exclusively by *Aspergilius parasiticus*. While the presence of *Aspergillus* in food products does not always indicate harmful levels of aflatoxin are also present, it does imply a significant risk in consumption.

Aflatoxins M1, M2 were originally discovered in the milk of cows which fed on mouldy grain. These compounds are products of conversion process in the animal's liver. However, aflatoxin M1 is present in the fermentation broth of *Aspergillus parasiticus*.

The aflatoxins can't be destroyed by thermal procedures and form this reason can produce acute necrosis, cirrhosis and liver carcinoma.

Nowadays, conventional methods for aflatoxin B1 detection mainly include thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), mass spectrometry (MS), radioimmunoassay (RIA), immunoaffinity column assay (ICA) and enzyme-linked immunosorbent assay (ELISA). In the last years, the quartz crystal microbalance technique which offers some advantages, as following: high sensitivity, real-time output, cost-effectiveness, and experimental simplicity, represented a real interest for the researchers world [1]. In brief, the quartz crystal surface was coated with a self-assembled monolayer of methanethiol to obtain a better sensitivity. On the future, the calibration curve can be used to determine the aflatoxins concentration in any type of milk (human milk, powder milk or fresh milk).

2. EXPERIMENTAL

2.1. REAGENTS AND MATERIALS

For this study, some materials and reagents, as aflatoxin standards from Charm Science, methanol (ICCF Bucharest) and methanethiol (Aldrich) are used. The standards were diluted and 5 samples with different concentrations were obtained. These samples were deposited as a thin layer on the crystal surface coated with self-assembled monolayer.

2.2. ANALYTICAL TECHNIQUES

Quartz Crystal Microbalance is an analytical device that are capable to monitoring some chemical species continuous and reversible [2].

A quartz crystal microbalance (QCM) measures a mass per unit area by measuring the change in frequency of a quartz crystal resonator. The resonance is disturbed by the addition or removal of a small mass due to oxide growth/decay or film deposition at the surface of the acoustic resonator [2, 3]. The QCM can be used under vacuum, in gas phase ("gas sensor", first use described by King) and more recently in liquid environments.

It is useful for monitoring the rate of deposition in thin film deposition systems under vacuum. In liquid, it is highly effective at determining the affinity of molecules (proteins, in particular) to surfaces functionalized with recognition sites. Larger entities such as viruses or polymers are investigated, as well. Also QCM has been used to investigate interactions between biomolecules [3]. Frequency measurements are easily made to high precision (discussed below); hence, it is easy to measure mass densities down to a level of below 1 $\mu g/cm^2$.

In this study, the QCM apparatus, *Stanford Research System – QCM 200*, with a 5 MHz oscillator and electrodes AT-cut, was used. This device is based on piezoelectric characteristics of the quartz crystal and uses many electrodes (CrAU, TiAu, TiPt).

In experimental study, for aflatoxin analysis, a TiAu electrode was used. The SAM (self-assembled monolayer) was obtained by adding 1 mL methanethiol (CH_2-CH_3-SH) 1% (Fig.3). The process was completed after 1.5 minutes, and then the new electrode, TiAu/thiol was dried. All experiments were performed with duplicate measurements and the experimental temperature was controlled at 25^0C.

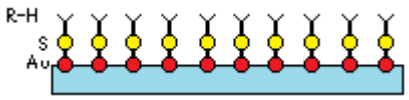

Fig. 3. Self-assembled monolayer – methanethiol on TiAu.

3. RESULTS AND DISCUSSION

The samples were deposited successive on the electrode surface and the frequency (F) and the resonance (R) were measured. Also, the software shown the variation in time of these two parameters and recorded the measured values (Fig.4) and the values of other calculated parameters (ΔF, ΔR, Δm) are presented in Table 1. The concentration of samples ranging from 0 to 4 ppb (Table 1) and the deposited amount of samples increase from 3.913 (μg/cm^2) to 11.542 (μg/cm^2).

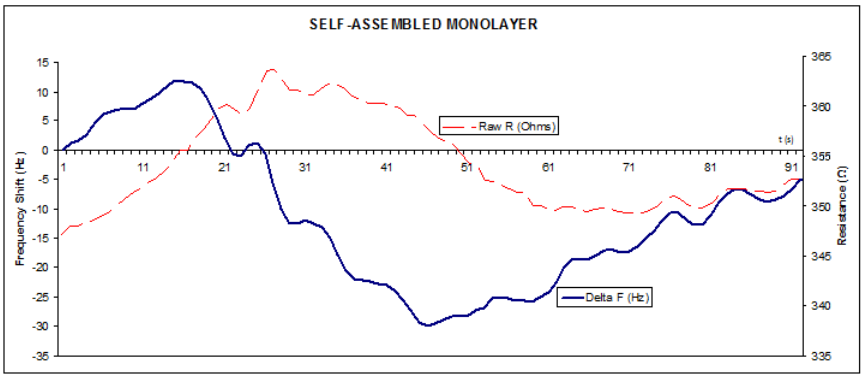

Fig. 4. Frequency shift and resistance of SAM on TiAu.

Table 1. The calculated parameters by QCM – software

C (ppb)	0	0.25	0.5	1.0	2.0	4.0
ΔF (Hz)	221.5	254.0	420.5	599.7	633.3	653.3
R (Ω)	271.39	293.11	338.53	420.17	436.95	423.36
Δm (μg/cm^2)	3.913	4.488	7.429	10.595	11.189	11.542

The research shown that, after 40 seconds, an exponential evolution of parameters (ΔF, R, Δm) depending on the concentration of Aflatoxin B1 from sample (e.g. Fig. 5), was observed.

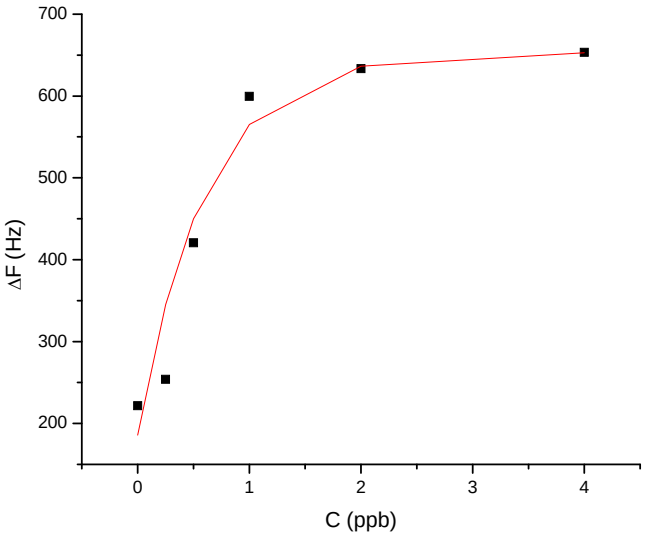

Fig. 5. Dependence between frequency and concentration of Aflatoxin B1.

Other parameters (R, Δm) can be plotted as dependence between them and Aflatoxin B1concentration from sample.

After plotting with software Origin 7, the correlation equation of frequency with concentration, was obtained.

$y = y_0 + A \cdot e^{-x/t}$ (1)

$y_0 = 653.3$ (2)

$A = -467.558$ (3)

$t = 0.59976$ (4)

$\Delta F = 653.3 - 467.558 \cdot e^{-C/0.59976}$ (5)

The obtained error was 11.539%.

The typical characteristics of frequency response of QCM in time at different Aflatoxin B1 concentrations from sample are represented in Fig. 6.

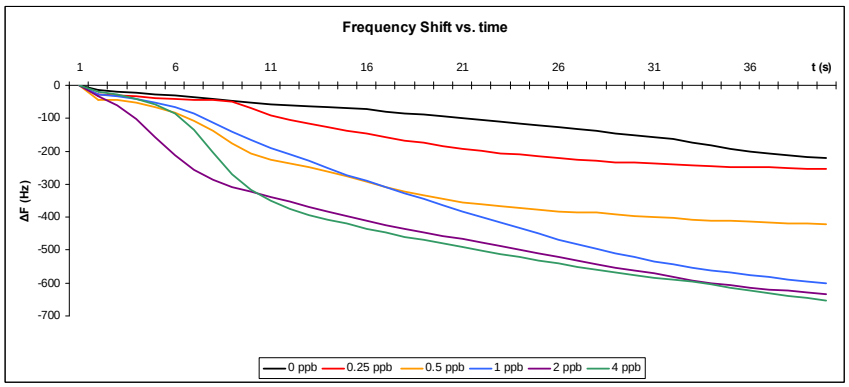

Fig. 6. Frequency shift in time for all samples.

4. CONCLUSIONS

The present work demonstrated an ultrasensitive technique for the determination of Aflatoxin B1 from various products. This sensitive method with real-time output and low cost used quartz crystal microbalance (QCM). Experimental data shown that the frequency response plotted vs. Aflatoxin B1 concentration in sample or vs. time at different concentration, determine mass changes as a result of frequency changes. Experimental studies provide that QCM technique is a promising alternative for the detection of aflatoxins and other mycrotoxins, especially form milk.

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