Journal of Zhejiang University-SCIENCE C (Computers & Electronics)
ISSN 1869-1951 (Print); ISSN 1869-196X (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

A micro-machined thin film electro-acoustic biosensor for detection of pesticide residuals*

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Received Oct. 16, 2013; Revision accepted Mar. 6, 2014; Crosschecked Apr. 11, 2014

Abstract: Increasing awareness concerning food safety problems has been driving the search for simple and efficient biochemical analytical methods. In this paper, we develop a portable electro-acoustic biosensor based on a film bulk acoustic resonator for the detection of pesticide residues in agricultural products. A shear mode ZnO film bulk acoustic resonator with a micro-machining structure was fabricated as a mass-sensitive transducer for the real-time detection of antibody-antigen reactions in liquids. In order to obtain an ultra-low detection level, the artificial antigens were immobilized on the sensing surface of the resonator to employ a competitive format for the immunoassays. The competitive immunoreactions can be observed clearly through monitoring the frequency changes. The presence of pesticides was detected through the diminution of the frequency shift compared with the level without pesticides. The limit of detection for carbaryl (a widely used pesticide for vegetables and crops) is 2×10^{-10} M. The proposed device represents a potential alternative to the complex optical systems and electrochemical methods that are currently being used, and represents a significant opportunity in terms of simplicity of use and portability for on-site food safety testing.

Key words: Biosensors, Electro-acoustic resonator, Pesticide residues, Immunoreactions
doi:10.1631/jzus.C1300289 **Document code:** A **CLC number:** TP212.3

1 Introduction

With the vast and irrational use of pesticides, the pesticide residues in farm products have become one of the most serious problems affecting human health. In the past, the standard methods for detecting residual pesticides were liquid or gas chromatographic (GC) analyses. These methods can offer precise and reliable analytical results, but they require expensive instrumentation, long test time, and professional operators. In practice, rapid and simple operating methods are highly desirable to allow for the detection of pesticide residues in farm products before they are distributed to consumers. Therefore, portable biosensors that can be applied for on-site detection have become one of the current research hotspots. Based on various signal transduction mechanisms, the current existing pesticide biosensor devices primarily use enzyme-linked immunosorbent assay (ELISA) (Deng *et al.*, 2003), surface plasmon resonance (SPR) (Narsaiah *et al.*, 2012), electrochemical enzyme sensor (Pundir and Chauhan, 2012), and quartz crystal microbalance (QCM) (March *et al.*, 2009; Erbahar *et al.*, 2012). These devices allow for sensitive detection with a limit-of-detection (LOD) range of 10^{-7}–10^{-8} M. However, they can hardly satisfy the

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* Project supported by the National Science Foundation of China (No. 61101027), Qingdao Science and Technology Program (No. 12-1-4-6-(8)-jch), Shangdong Provincial Young and Middle-Aged Scientists Research Awards Fund (No. BS2012DX032), Shandong Provincial Natural Science Foundation (No. ZR2013FM018), and SDUST Research Funds (Nos. 2011KYJQ101 and 2012KYTD103)

increasing requirements of low cost, being easy to use, and providing high-through detection, due to their large sizes and/or the complex signal detection methods utilized such as the use of a position sensitive laser.

To overcome these disadvantages, an electro-acoustic biosensor based on a film bulk acoustic resonator (FBAR) was developed (Wingqvist, 2010; Chen *et al.*, 2011; 2012; Katardjiev and Yantchev, 2012). With micro-electromechanical system (MEMS) technology, FBAR enables the integration of the sensor array with the electronic circuits and the microfluidic devices into a very small chip. For the applications involving biochemical sensing, FBAR works as a mass-sensitive transducer similar to a traditional QCM. However, the significant improvement of FBAR over QCM is the use of a 1–2 μm thick piezoelectric thin film, which provides a fundamental resonant frequency of more than 2 GHz and thereby creates a mass sensitivity of more than 1000 Hz·cm^2/ng (Voiculescu and Nordin, 2012). This type of performance is an improvement of 1000 times when compared with those of a typical QCM, making FBAR ideal for ultralow concentration analytical applications.

In this paper, we realize a highly sensitive, rapid, and convenient detection of pesticide residues in farm products by combing the high sensitivity of FBARs and the specificity of antibody-antigen reactions. Considering that the pesticide molecule is very small, we employ a competitive assay format through immobilization of the artificial antigens on the FBAR sensing surface. In this way, an ultra-low detection level is obtained.

2 Experimental

2.1 Structure and fabrication of the FBAR biosensor

Fig. 1 shows the basic configuration of the FBAR biosensor. The mass-sensitive FBAR with a composite structure was fabricated using a standard MEMS process. The 800-μm-thick ZnO film with (002) texture is deposited on a 0.6-μm-thick silicon nitride diaphragm by a magnetron sputtering method (Chen *et al.*, 2010). On the other side of the silicon wafer, the silicon is chemically etched by KOH to isolate the ZnO film from the substrate and also create a testing channel for the liquid to be analyzed. For the modification of the biomolecules, a 20-nm-thick Au layer was deposited on the bottom of the completed testing channel. Two parallel electrodes with a gap of 10 μm were placed on the same side of the ZnO film to excite the shear mode acoustic wave. The feasibility of this lateral excitation method has been verified by theoretical and experimental analyses reported in our previous paper (Chen *et al.*, 2010). Due to the minimal damping of the shear acoustic wave in the adjacent liquid medium, the shear mode resonance has a relatively high Q-factor in liquids and therefore is more suitable for biological sensing compared with the longitudinal mode approach.

Fig. 2 shows the photographs of the individual FBAR biosensor and the fabricated circuit board used for testing. The FBAR biosensor was mounted on a printed circuit board (PCB) and wire bonded to an oscillation circuit. The whole area of the PCB was only 2 cm×1.5 cm. The circuit output was connected to a spectrum analyzer (Agilent 8714 ET) via a standard 50 Ω cable to measure the resonant frequency of the FBAR. In the test, the circuit board was turned over so that the testing channel was loaded with the flowing solution. A syringe pump (Longer LSP01-1A) was used to deliver the analyte liquids.

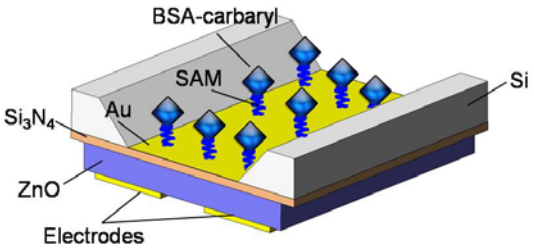

Fig. 1 Basic configuration of the FBAR biosensor

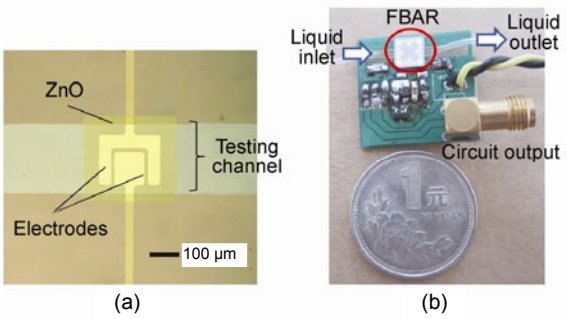

Fig. 2 The top view microphotograph (a) and the PCB of the fabricated FBAR biosensor (b)

2.2 Modification of artificial antigens

For a demonstration, carbaryl, which is one of the most widely applied pesticides for vegetables and crops, was chosen as the target in this experiment. For the competitive immunoassays, the artificial antigens, and bovine serum albumin-carbaryl (BSA-carbaryl) conjugates (AbD Serotec), were immobilized on the FBAR sensing surface through the formation of a self-assembled monolayer (SAM). At the beginning, the Au surface on the bottom of the testing channel was cleaned with a piranha solution (7:3, 98% H_2SO_4/30% H_2O_2) and immersed into 3-Mercaptopropionic acid (MPA, Sigma-Aldrich) solution for 1 h at 30 °C to form the SAM. After rinsing with ethanol and water, the SAM was treated with an ethanolic solution mixture of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma-Aldrich) and 0.5 M N-hydroxy sulfosuccinimide (NHS, Sigma-Aldrich) for 30 min. During this reaction, EDC converted the terminal carboxylic group into an N-hydroxysuccinimide ester, which covalently reacted with the lysine amine groups of the BSA-carbaryl conjugates. Then the active surface was washed with ethanol and dried with nitrogen gas. For the immobilization of BSA-carbaryl conjugates, the active surface was further immersed into 1 g/L BSA-carbaryl in a phosphate buffer solution (PBS, 10 mM, PH 7.4) for 1 h at 37 °C. Finally, 1% BSA in PBS was used to block the unreacted NHS-esters. After rinsing again with ethanol and distilled water, the FBAR biosensor was prepared and ready for test.

2.3 Competitive analysis using the FBAR biosensor

In performing the measurements, the analyte liquids were pushed into the testing channel and passed through the FBAR sensing surface by means of a syringe pump at a constant flow rate of 0.2 μL/min. The resonant frequency of the FBAR working in PBS (the background solution) was recorded as the baseline frequency. The solution of glycine-HCl buffer (PH 2.3) was used to regenerate the FBAR biosensor.

The sensing principle of the FBAR biosensor in this study is based on the competitive binding of artificial antigens and pesticide molecules (haptens) to the antibodies. First, the 10 μg/L carbaryl monoclonal antibodies (MAb, AbD Serotec) solution was injected into the testing channel to initiate the antibody-antigen reactions. As the antibodies bind to the immobilized artificial antigens on the FBAR surface, the resonant frequency is decreased caused by the increasing mass. During the antibody-antigen reactions, the resonant frequency of the FBAR was continuously monitored and the time evolution was obtained by plotting the frequency shift versus time. For the detection of pesticides, the mixtures of the carbaryl-MAb solution and the standard carbaryl solutions (10^{-12}–10^{-6} M) were injected into the testing channel. Given that the pesticide molecules compete with the artificial antigens for the binding sites on the antibodies, the detection of pesticides can be carried out by following the diminution of the frequency shift from the levels found in their absence.

3 Results and discussion

3.1 Resonance performances of the FBAR biosensor

The FBAR works as a mass-sensitive transducer for the immunoassay. Fig. 3 shows the admittance curves of the FBAR working in the background solution of PBS before and after modification. Before the modification of the artificial antigens, the bare FBAR shows a clear resonance at 1730.1 MHz with a Q-factor of 470 in PBS. The Q-factor was calculated from the admittance curves using the following equation (Satoh *et al.*, 2005):

$$Q=\left|\frac{f}{\mathrm{FWHM}}\right|_{f=f_R}, \tag{1}$$

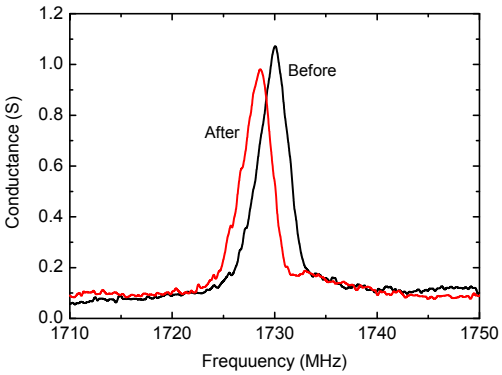

Fig. 3 The conductance curves of the FBAR working in the background solution of PBS before and after immobilization of artificial antigens

where FWHM is the full wave at half maximum of the conductance curve at resonant frequency f_R.

After the modification of artificial antigens, the resonant frequency of the FBAR decreased by 1.42 MHz due to the mass-sensitive mechanism. This frequency decrease indicates that the artificial antigens have been efficiently bound to the Au surface via the SAM.

3.2 Real-time monitoring of the competitive immunoreactions

As the pesticide molecule is very small, the direct binding of the pesticide molecules to the antibodies cannot lead to a measurable mass change for the mass-sensitive transducer when the pesticide concentration is very low (Přibyl *et al.*, 2003). In order to increase the sensitivity of the FBAR biosensor, the competitive assay format was employed in this study. For this purpose, the artificial antigens (BSA-carbaryl) were immobilized on the FBAR surface. Based on the mass-sensitive principle, the binding of antibodies to artificial antigens will reduce the resonant frequency of the FBAR. However, when the pesticide molecules and the antibodies pass through the FBAR sensing surface together, the pesticide molecules, as the haptens, will compete with the artificial antigens for the binding sites on the antibodies. The formation of the antibody-antigen complexes in the flowing solution results in a loss of the bound antibodies onto the FBAR sensing surface. Consequently, the competitive immunoreactions can be traced, in real-time and in-situ, by measuring the frequency shifts caused by the deposited antibodies over the FBAR surface.

Fig. 4 shows the typical time-dependent frequency profile when a single MAb solution (10 μg/L) and the mixed solution of pesticide and MAb were injected into the testing channel of the FBAR biosensor. After each injection of the solution, the resonant frequency was decreased rapidly to a steady value. In the case of single MAb solution, the frequency profile exhibits a maximum change (Δf_{max}= 2180 kHz), corresponding to the maximum antibodies bound to the immobilized artificial antigens as shown by inset I in Fig. 4b. In the presence of pesticide molecules, the frequency shifts of the FBAR were inversely proportional to the pesticide concentrations in the mixture solution because the pesticide molecules bound to the antibodies prevent the antibodies from binding to the artificial antigens as shown by inset II in Fig. 4b. Therefore, the competitive immunoreactions can be clearly observed through monitoring the frequency changes of the FBAR.

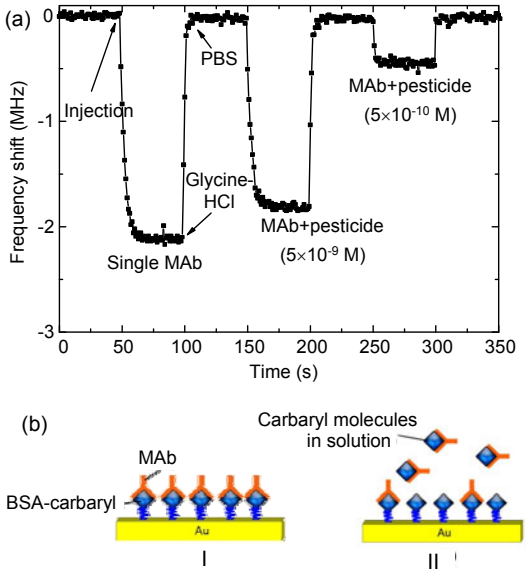

Fig. 4 (a) A typical time-dependent frequency profile when the single MAb solution (10 μg/L) and the mixed solution of pesticide and MAb were injected into the testing channel of the FBAR biosensor; (b) The schematics of competitive immunoreactions (I, in single MAb solution; II, in the mixed solution)

In addition, after the treatment with glycine-HCl buffer and PBS, the resonant frequencies were increased to the baseline frequency again, indicating that the association of the antibody-hapten conjugates on the FBAR surface was completely broken. These results confirm that a glycine-HCl buffer is an appropriate regenerant for the FBAR biosensor.

3.3 Standard curve

To determine the pesticide concentrations, the frequency shifts were used to plot the standard calibration curve as shown in Fig. 5. The data in this figure was calculated according to the average values of the 10 measured results for each concentration. Then, the frequency shifts were normalized as the response of the FBAR biosensor by expressing experimental frequency changes as $100\Delta f/\Delta f_{max}$, where Δf_{max} is the maximum frequency shift (in single MAb solution). The relationship between the frequency

shifts and the concentrations shows a decreasing sigmoidal shape, which is a typical characteristic of the binding inhibition immunoreactions (Přibyl *et al.*, 2003; March *et al.*, 2009). In the concentration range of 1×10^{-10}–1×10^{-8} M, a near-linear relationship on a semi-logarithmic scale was noted between the pesticide concentration and the sensor response. The concentration giving half of the maximum response (I_{50}), which is generally accepted as an estimate of the competitive immunosensor sensitivity, was 1.4×10^{-9} M for carbaryl. The lLOD, defined as the concentration that provided 90% of the maximum response (I_{90}), was 2×10^{-10} M. As shown in Table 1, the LOD value of the FBAR biosensor is two orders of magnitude lower than that of the QCM biosensors (Kim *et al.*, 2007; March *et al.*, 2009; Erbahar *et al.*, 2012), and comparable to, or lower than, the values of the optical methods, ELISA, and electrochemical methods (Jiang *et al.*, 2008; Narsaiah *et al.*, 2012). However, in our case, we are able to employ a significantly smaller sensor size and simpler operation in the packed system to realize this real-time detection.

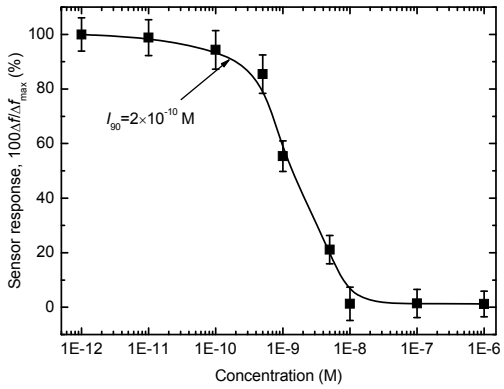

Fig. 5 The standard calibration curve of the FBAR biosensor for carbaryl (Δf_{max} is the maximum frequency shift, in single MAb solution)

3.4 Cross-reactivity

The selectivity of the FBAR biosensor was evaluated by comparing its response with several related compounds. The cross-reactivity (CR) was defined as the percentage ratio between the I_{50} value of the target analyte and the I_{50} value of the cross-reacting compounds as follows (March *et al.*, 2009):

$$\mathrm{CR}=\frac{I_{50}(\text{target})}{I_{50}(\text{related analytes})}\times100\%. \tag{2}$$

Table 2 shows the cross-reactivity of the FBAR biosensor. No related compounds gave a cross-reactivity higher than 0.3%, and three compounds gave values lower than 0.1%. These results indicated that a high selectivity was achieved using the FBAR biosensor.

Table 2 Cross-reactivity (CR) of the FBAR biosensor

Compound	CR (%)
Carbaryl	100
Carbofuran	0.21
1-Naphtol	0.27
Methiocarb	0.16
Bendiocarb	<0.10
Propoxur	<0.10
Trichlopyr	<0.10

3.5 Stability of the FBAR biosensor

To investigate the stability of the FBAR biosensor, the measurements were performed after several days using the devices stored in nitrogen at 4 °C. Fig. 6a shows the measured frequency shifts of the FBAR biosensor exposed to a single MAb solution and the mixed solutions of pesticide and MAb during

Table 1 Comparison between the FBAR biosensor and other methods for pesticide detection

Method	Pesticide(s)	LOD (M)	Working range (M)	Reference
ELISA	Chlorpyrifos-methyl	10^{-9}	3×10^{-9}–2×10^{-4}	Qian *et al*. (2009)
SPR	Carbaryl	7×10^{-10}	1.3×10^{-9}–1.7×10^{-8}	Mauriz *et al*. (2006)
Amperometric electrochemical	Malathion	3×10^{-9}	2.1×10^{-8}–5.3×10^{-6}	Dua *et al*. (2010)
Voltammetry electrochemical	Methyl parathion	10^{-12}	10^{-10}–10^{-7}	Viswanathan *et al*. (2009)
Potentiometric electrochemical	Chlorpyrifos	2×10^{-10}	10^{-11}–10^{-4}	Lee *et al*. (2002)
QCM	TCP	1.8×10^{-8}	3.4×10^{-8}–2.1×10^{-7}	March *et al*. (2009)
	Carbaryl	1.5×10^{-8}	7.5×10^{-8}–2.7×10^{-7}	
FBAR biosensor	Carbaryl	2×10^{-10}	10^{-10}–10^{-8}	This paper

10 days. The frequency shifts decreased slightly as time went on, which can be ascribed to the weakened immune activity of the artificial antigens immobilized on the FBAR sensing surface. Furthermore, the frequency shifts measured on different days were normalized by the original values measured on the first day (Δf_0) to show the relative changes. As shown in Fig. 6b, there was a similar dependence of the normalized frequency shifts on the time for all the solutions, indicating that the decreased frequency shifts of the FBAR biosensor rarely affected the sensing response of the FBAR biosensor.

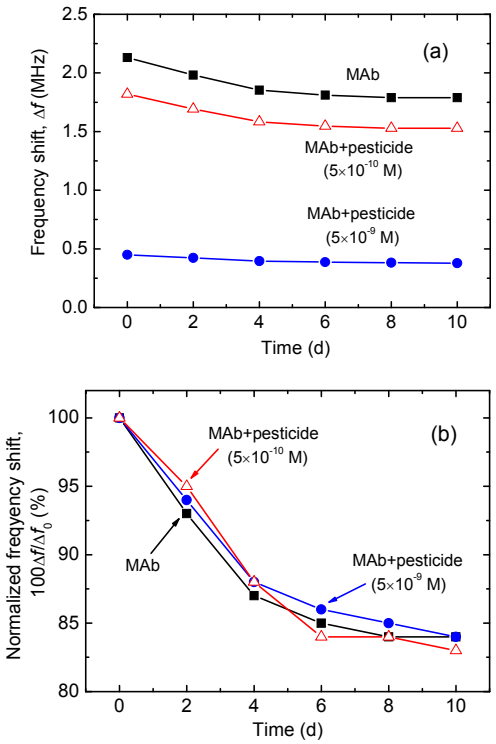

Fig. 6 The frequency shifts of the FBAR biosensor exposed to a single MAb solution and the mixed solutions of pesticide and MAb during 10 days

(a) Original measured values; (b) Values normalized by the original value measured on the first day (Δf_0)

4 Conclusions

We developed a novel FBAR biosensor modified with artificial antigens and verified that it could be used as a feasible tool for the detection of pesticide residues. The presence of pesticides can be detected according to the diminution of the frequency shift compared with the level without pesticides. Based on the mass-sensitive FBAR, an ultralow LOD of 2×10^{-10} M for carbaryl was achieved by employing the competitive assay format. At the same time, the FBAR biosensor exhibits high specificity and acceptable stability. The repeated use of the FBAR biosensor was possible after regeneration using a glycine-HCl buffer. In particular, the economical FBAR biosensor represents a potential alternative to the complex optical systems and electrochemical methods, and a significant promise in terms of simplicity of use and portability for on-site food safety testing in the market and at home. For future developments, the FBAR biosensor arrays employing different bio-recognition receptors will be fabricated to detect multiple pesticide residuals simultaneously.

References

Chen, D., Wang, J.J., Xu, Y., *et al.*, 2010. The pure-shear mode solidly mounted resonator based on c-axis oriented ZnO film. *Appl. Surf. Sci.*, **256**(24):7638-7642. [doi:10.1016/j.apsusc.2010.06.020]

Chen, D., Wang, J.J., Liu, Q.X., *et al.*, 2011. Highly sensitive ZnO thin film bulk acoustic resonator for hydrogen detection. *J. Micromech. Microeng.*, **21**(11):1150181-1150187. [doi:10.1088/0960-1317/21/11/115018]

Chen, D., Wang, J.J., Xu, Y., *et al.*, 2012. A thin film electroacoustic enzyme biosensor allowing the detection of trace organophosphorus pesticides. *Anal. Biochem.*, **429**(1):42-44. [doi:10.1016/j.ab.2012.07.002]

Deng, A.P., Himmelsbach, M., Zhu, Q.Z., *et al.*, 2003. Residue analysis of the pharmaceutical diclofenac in different water types using ELISA and GC-MS. *Environ. Sci. Technol.*, **37**(15):3422-3429. [doi:10.1021/es0341945]

Dua, D., Yea, X., Caib, J., *et al.*, 2010. Acetylcholinesterase biosensor design based on carbon nanotube-encapsulated polypyrrole and polyaniline copolymer for amperometric detection of organophosphates. *Biosens. & Bioelectron.*, **25**(11):2503-2508. [doi:10.1016/j.bios.2010.04.018]

Erbahar, D.D., Gurol, I., Gumus, G., *et al.*, 2012. Pesticide sensing in water with phthalocyanine based QCM sensors. *Sens. Actuat. B*, **173**:562-568. [doi:10.1016/j.snb.2012.07.041]

Jiang, X.S., Li, D.Y., Xu, X., *et al.*, 2008. Immunosensors for detection of pesticide residues. *Biosens. & Bioelectron.*, **23**(11):1577-1587. [doi:10.1016/j.bios.2008.01.035]

Katardjiev, I., Yantchev, V., 2012. Recent developments in thin film electro-acoustic technology for biosensor applications. *Vacuum*, **86**(5):520-531. [doi:10.1016/j.vacuum.2011.10.012]

Kim, N., Park, I.S., Kim, D.K., 2007. High-sensitivity detection for model organophosphorus and carbamate pesticide with quartz crystal microbalance-precipitation sensor.

Biosens. & Bioelectron., **22**(8):1593-1599. [doi:10.1016/j.bios.2006.07.009]

Lee, H.S., Kim, Y.A., Cho, Y.A., *et al.*, 2002. Oxidation of organophosphorus pesticides for the sensitive detection by a cholinesterase-based biosensor. *Chemosphere*, **46**(4):571-576. [doi:10.1016/S0045-6535(01)00005-4]

March, C., Manclús, J.J., Jiménez, Y., *et al.*, 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]

Mauriz, E., Calle, A., Abad, A., *et al.*, 2006. Determination of carbaryl in natural water samples by a surface plasmon resonance flow-through immunosensor. *Biosens. & Bioelectron.*, **21**(11):2129-2136. [doi:10.1016/j.bios.2005.10.013]

Narsaiah, K., Jha, S.N., Bhardwaj, R., *et al.*, 2012. Optical biosensors for food quality and safety assurance—a review. *J. Food Sci. Technol.*, **49**(4):383-406. [doi:10.1007/s13197-011-0437-6]

Přibyl, J., Hepel, M., Halámek, J., *et al.*, 2003. Development of piezoelectric immunosensors for competitive and direct determination of atrazine. *Sens. Actuat. B*, **91**(1-3):333-341. [doi:10.1016/S0925-4005(03)00107-2]

Pundir, C.S., Chauhan, N., 2012. Acetylcholinesterase inhibition-based biosensors for pesticide determination: a review. *Anal. Biochem.*, **429**(1):19-31. [doi:10.1016/j.ab.2012.06.025]

Qian, G.L., Wang, L.M., Wu, Y.R., *et al.*, 2009. A monoclonal antibody-based sensitive enzyme-linked immunosorbent assay (ELISA) for the analysis of the organophosphorous pesticides chlorpyrifos-methyl in real samples. *Food Chem.*, **117**(2):364-370. [doi:10.1016/j.foodchem.2009.03.097]

Satoh, Y., Nishihara, T., Yokoyama, T., *et al.*, 2005. Development of piezoelectric thin film resonator and its impact on future wireless communication systems. *Jpn. J. Appl. Phys.*, **44**(5A):2883-2894. [doi:10.1143/JJAP.44.2883]

Viswanathan, S., Radecka, H., Radecki, J., 2009. Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA. *Biosens. & Bioelectron.*, **24**(9):2772-2777. [doi:10.1016/j.bios.2009.01.044]

Voiculescu, I., Nordin, A.N., 2012. Acoustic wave based MEMS devices for biosensing applications. *Biosens. & Bioelectron.*, **33**(1):1-9. [doi:10.1016/j.bios.2011.12.041]

Wingqvist, G., 2010. AlN-based sputter-deposited shear mode thin film bulk acoustic resonator (FBAR) for biosensor applications—a review. *Surf. Coat. Technol.*, **205**(5):1279-1286. [doi:10.1016/j.surfcoat.2010.08.109]