5th INTERNATIONAL SCIENTIFIC CONFERENCE

OTEH 2012

ON DEFENSIVE TECHNOLOGIES

PROCEEDINGS

Belgrade, 18 – 19 september 2012

Publisher
The Military Technical Institute
Ratka Resanovića 1, 11030 Belgrade

Publisher's Representative
Doc. Col **Zoran Rajić,** PhD (Eng)

Editor
Jovan Isaković, PhD (Eng)

Technical Editing
Dragan Knežević
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Graphic Editing
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Printing
300 copies

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

623.4/.7(082)(0.034.2)
66.017/.018:623(082)(0.034.2)

NAUČNO-stručni skup iz oblasti odbrambenih tehnologija (5 ; 2012 ; Beograd)
Proceedings [Elektronski izvor] / 5th International Scientific Conference on Defensive Technologies, OTEH 2012, Belgrade, 18-19 september 2012 ; [organized by Military Technical Institute, Belgrade ; editor Jovan Isaković]. - Belgrade : Military Technical Institute, 2012 (Beograd : Military Technical Institute). - 1 elektronski optički disk (CD-ROM) ; 12 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž 300. - Bibliografija uz svaki rad.

ISBN 978-86-81123-58-4
1. Vojnotehnički institut (Beograd)
a) Војна техника - Зборници b) Технички материјали - Зборници
COBISS.SR-ID 193395212

5th INTERNATIONAL SCIENTIFIC CONFERENCE

OTEH 2012

ON DEFENSIVE TECHNOLOGIES

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Ministry of Defence

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ON DEFENSIVE TECHNOLOGIES
OTEH 2012
Belgrade, Serbia, 18 – 19 September 2012

DETECTION OF TARGET SUBSTANCES USING AFFINITY-BASED MEMS/NEMS SENSORS: A PROBLEM OF SELECTIVITY

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Abstract: *Affinity-based micro/nanosensors intended for detection of various chemical or biological agents in a liquid or gaseous environment have a great potential for both civilian and military applications. Selective detection of a target substance is based on highly specific binding of its particles to the functionalized surface of the sensing element. However, samples taken from the environment often contain substances other than the target, which can also bind with a certain affinity to the same functionalizing layer or binding sites, thus affecting the sensor's response. In this paper we analyze the influence of limited selectivity of a sensor on its time response. The results show that binding of a non-target substance can cause a significant change in both the equilibrium value and the rate of the sensor's response. The presented analysis enables improvement of accuracy of determination of the target substance concentration in detection methods based on time domain measurements of the sensor's output signal. It is also useful for development of methods for simultaneous detection of multiple substances.*

Key words: *Micro/nanoelectromechanical sensor, chemical agent, biological agent, sensor selectivity.*

1. INTRODUCTION

Chemical and biological agents, in the form of weapons employed by terrorists or by an enemy, toxic spills or naturally occurring pandemics, pose a significant risk to the health and safety of people in the modern world. Globalization and enhanced mobility, as well as the progress in chemical and biotechnological expertise, facilitate production and transport of hazardous substances and spreading of contagious diseases. Therefore rapid and accurate detection of the presence of chemical and biological threat agents is critical for timely warning and effective response.

A typical high performance chemical or biological detection system used today requires time-consuming, labor-intensive and costly laboratory processes. For example, airborne particles from an environment are first captured onto solid filters, which are then collected manually and transported to laboratories for analysis, where expensive laboratory equipment is used. This approach is inadequate if real-time in situ detection of chemical and biological agents is needed.

In order to protect both population and troops from harmful agents it is extremely important to have chemical and biological sensors of high sensitivity, capable of rapid detection and identification of chemicals or pathogens, as well as to obtain the data from the sensors as soon as possible. Therefore, the sensors have to be portable, i.e. of small dimensions and lightweight, or to be distributed in the field, which would require their autonomous operation and wireless communication for remote real-time or near-real time readout of measured data and sending of warning reports. Rapid sensor regeneration is also necessary. An integrated system of sensors is desirable, enabling simultaneous detection of multiple threat agents, such as chemicals, bacteria, spores, viruses, toxins etc.

Sensors fabricated using MEMS/NEMS (micro/nano-electromechanical system) technologies intended for detection of various chemical or biological agents in a liquid or gaseous environment are highly sensitive and enable real-time measurements [1]. They are also low power, compact and lightweight devices, and therefore portable, making in situ measurements possible. MEMS/NEMS sensors are capable of detecting minuscule concentrations of target substances in extremely small volumes of samples. Additionally, the MEMS technology enables integration of transducers with read-out and

signal processing electronic circuits, decreasing the device size, detection time and cost and making devices more reliable. Simultaneous detection of a larger number of agents using integrated arrays of MEMS/NEMS sensors is also possible [2, 3]. Therefore, chemical and biological MEMS/NEMS sensors have a great potential for both civilian and military applications [4].

In a large group of chemical and biological micro/nano-sensors, known as affinity-based, surface-based or adsorption-based, a selective detection of a target substance is based on high-affinity binding (i.e. adsorption) of its particles to the functionalized surface of the sensing element. This binding changes some optical, mechanical or electrical parameter of the sensing element, which is converted to the sensor's output signal.

Samples taken from the environment often contain substances other than the target, which can also bind with a certain affinity to the same functionalizing layer or molecules, thus affecting the sensor's performance. In affinity-based sensors various methods (surface modifications, blocking agents etc.) are applied in order to minimize the unwanted binding of other substances to the sensing surface, thus improving sensor selectivity. However, in biosensors it is sometimes necessary to use functionalizing biomolecules that have multiple binding sites (called receptor sites) with the affinity for binding of different biomolecular species. Different molecules that bind to functionalizing molecules can be of similar size and mass as the target molecules and cannot be eliminated from the sample. A similar problem of functionalizing layer selectivity can also exist in gas sensors.

In this paper we analyze the influence of limited selectivity of an affinity-based MEMS/NEMS sensor on its time response.

2. THEORY

In affinity-based methods for detection of chemicals or biological specimens it is highly desirable that only the target entities (atoms, molecules or microorganisms) bind to the sensing surface. However, this idealized situation is more or less compromised in the reality. We analyze the situation when one molecular species existing in the sample together with the target molecules, can also bind to the molecules used for the sensor functionalization (Picture 1). We will call them the interferer molecules.

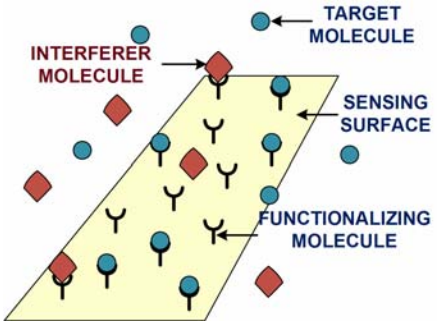
Under the following assumptions:

- in the sensor's reaction chamber reversible binding reactions occur between each molecular species (target and interferer) and functionalizing molecules, which does not alter any of reacting molecules
- each functionalizing molecule has one type of binding sites for one molecular species, and all functionalizing molecules are equivalent
- only one molecule can be bound to a functionalizing molecule at any time
- the target and interferer molecules do not interact with each other
- the binding-unbinding reactions do not change significantly the concentrations of unbound target and interferer molecules in the sensor's reaction chamber (the ambient analyte conditions [5] or the system with steady flow of the sample through the reaction chamber [6])
- transport of both the target and the interferer molecules to the sensing surface is fast compared to the binding reactions

the rates of change of the numbers of molecules bound to the sensing surface are given by the system of two differential equations

$$\begin{aligned} dN_T/dt &= k_{fT}C_T(N_m - N_T - N_i) - k_{rT}N_T \\ dN_i/dt &= k_{fi}C_i(N_m - N_T - N_i) - k_{ri}N_i \end{aligned}, \qquad (1)$$

where N_T is the instant number of bound target molecules, C_T, k_{fT}, and k_{rT} are the concentration in the sample, the association rate constant and the dissociation rate constant of the target molecules, respectively, and $N_m=n_mA$ is the number of functionalizing molecules (i.e. of binding sites for each molecular species) on the surface of area A (n_m is the binding sites surface density). N_i, k_{fi}, k_{ri} and C_i are the parameters for interferer molecules, that correspond to previously mentioned target molecules parameters N_T, k_{fT}, k_{rT}, and C_T.



Picture 1. Illustration of a binding process of both the target and the interferer molecules to the functionalized sensor's surface

Eqs. (1) can be solved analytically, yielding the time evolution of the number of bound target and interferer molecules

$$\begin{aligned} N_T(t) &= N_{Te} + K_I \exp(-t/\tau_I) + K_{II} \exp(-t/\tau_{II}) \\ N_i(t) &= N_{ie} + K_{III} \exp(-t/\tau_I) + K_{IV} \exp(-t/\tau_{II}) \end{aligned}. \qquad (2)$$

After the transient period, whose duration is determined by the time constants τ_I and τ_{II}

$$\tau_{I,II} = 2\left[\frac{1}{\tau_T} + \frac{1}{\tau_i} \mp \sqrt{\left(\frac{1}{\tau_T} - \frac{1}{\tau_i}\right)^2 + 4\frac{N_{Te1}N_{ie1}}{\tau_T\tau_i N_m^2}}\right]^{-1} \qquad (3)$$

the equilibrium is established, characterized by the equilibrium numbers of bound molecules

$$N_{Te} = \frac{N_m k_{fT} C_T / k_{rT}}{1 + k_{fT} C_T / k_{rT} + k_{fi} C_i / k_{ri}}$$
$$N_{ie} = \frac{N_m k_{fi} C_i / k_{ri}}{1 + k_{fT} C_T / k_{rT} + k_{fi} C_i / k_{ri}} \quad (4)$$

The parameter $\tau_T=(k_{rT}+k_{fT}C_T)^{-1}$ is the time constant of establishment of the equilibrium number of bound target molecules $N_{Te1}=k_{fT}C_T N_m \tau_T$, for the case when only the target molecules exist in the sample and bind to sensing surface, while $\tau_i=(k_{ri}+k_{fi}C_i)^{-1}$ and $N_{ie1}=k_{fi}C_i N_m \tau_i$ are the corresponding parameters for the reversible binding process of only the interferer molecules. The coefficients K_{I}-K_{IV} are determined by the initial conditions $N_T(0)=N_i(0)=0$ and Eqs. (1) and (2) which must be satisfied for every t

$$K_4 = \left(k_{fi} C_i N_{Te} + \left(\frac{1}{\tau_i} - \frac{1}{\tau_I} \right) N_{ie} \right) \cdot \left(\frac{1}{\tau_I} - \frac{1}{\tau_{II}} \right)^{-1}$$
$$K_3 = -N_{Ce} - K_4$$
$$K_2 = K_4 \left(\frac{1}{\tau_{II}} - \frac{1}{\tau_i} \right) \cdot (k_{fi} C_i)^{-1} \quad (5)$$
$$K_1 = -N_{Te} - K_2$$

The biosensor's signal depends on the number of bound molecules of both kinds. For example, in the dynamic mode micro/nanocantilever sensors [7] or thin film bulk acoustic resonator (FBAR) sensors [8], as molecules bind to the immobilized functionalizing molecules, the increasing bound mass on the surface causes a decrease in the frequency of the natural mechanical resonance of the structure. The resonant frequency can be monitored as a direct indication of the presence of bound molecules and their quantity. When both the target and the interferer molecules bind to the sensing surface the measured signal is determined by the total mass of bound molecules

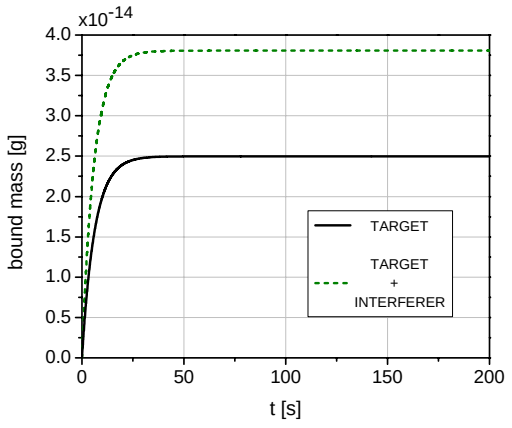
$$m_b = M_T N_T + M_i N_i \,, \quad (6)$$

where M_T and M_i are the molecular masses of the target analyte and the interferer, respectively. In this paper we will consider this case for the illustration of the influence of a non-ideal sensor's selectivity on its time response.

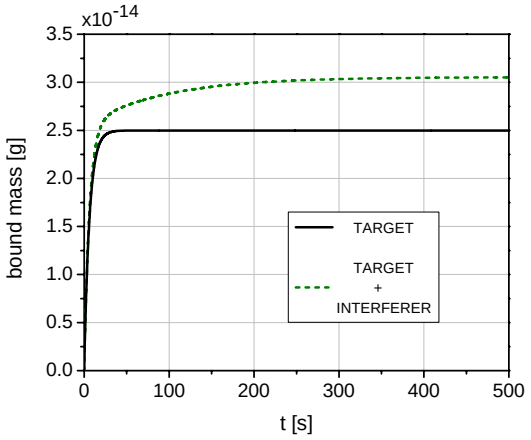
3. RESULTS AND DISCUSSION

The presented theory is used for the analysis of the influence of binding of interferer molecules on the biosensor's response which is proportional to the total mass of molecules bound to the sensing surface. The parameter values used in the analysis are realistic for biological samples and MEMS sensors [9]. The target molecules parameters are: $k_{fT}=8\cdot10^7$ $(\mathrm{Ms})^{-1}$, $k_{rT}=0.08$ s^{-1}, $C_T=1$ nM (unit 1 M=10^3 mol/m^3), $M_T=5$ kDa (unit 1 Da=$1.66\cdot10^{-27}$ kg). The binding sites surface density is $n_m=1\cdot10^{-11}$ Mm and the area of the sensing surface of MEMS device equals $A=1\cdot10^{-9}$ m^2. The three different interferer molecules species are considered separately, so that different effects of their binding are encompassed.

Picture 2 shows the time evolution of the mass of molecules bound to the sensing surface when ideal selectivity of the sensor is assumed, i.e. binding of the target molecules only (solid line), and when binding occurs of both the target and the interferer molecules with the certain affinity to the functionalizing molecules (dashed line).



a)



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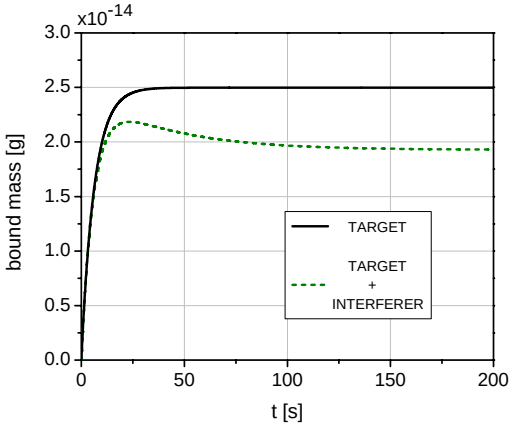

c)

Picture 2. The change of the bound mass in time, for three different types of interferer molecules: a) heavier than the target ($M_i=6M_T$), present in the same concentration in the sample, b) heavier than the target ($M_i=2M_T$) and in higher concentration ($C_i=2C_T$), c) lighter than target ($M_i=M_T/2$), in higher concentration ($C_i=2C_T$)

In the case shown in Picture 2a the interferer molecules are heavier than the target molecules (M_i=30 kDa), they are present in the sample in the same concentration (C_i=1·10^{-9} M) and have 10 times lower affinity for binding to the molecules used for functionalization of the sensor's surface (k_{fi}=8·10^6 (Ms)$^{-1}$, k_{ri}=0.08 s^{-1}). It can be observed that binding of the interferer molecules causes significant increase of the equilibrium value of the sensor's response compared to the case of ideal selectivity, while the influence on the transient is negligible.

Picture 2b shows the case of the interfering binding of molecules which are heavier (M_i=10 kDa) and present in the sample in the higher concentration (C_i=2·10^{-9} M) than the target particles, and which have more than 10 times lower binding affinity (k_{fi}=8·10^5 (Ms)$^{-1}$, k_{ri}=0.01 s^{-1}). In this case, apart from the increased equilibrium value of the resulting signal, the sensor's response speed is also significantly lower compared to the case of binding of only the target molecules.

The case shown in Picture 2c refers to the case of the interferer molecules which are lighter than the target molecules (M_i=500 Da). Their concentration in the sample is C_i=2·10^{-9} M, and their affinity for binding is more than two times lower (k_{fi}=8·10^6 (Ms)$^{-1}$, k_{ri}=0.02 s^{-1}) than the binding affinity of the target molecules for the same functionalizing molecules. Although the additional molecular species is bound, so the resulting signal corresponds to the sum of the masses of bound molecules of both kinds, it is lower than in the case of binding of only the target molecules. This is because the interferer molecules compete with the target molecules for the same functionalizing molecules, leading to the decrease of the number of bound target molecules compared to the case when there is no interferer molecules in the sample. It follows from Eqs. (4) and (6) that the suppression of the target molecules binding will result in the decrease of the total equilibrium bound mass if $M_i<M_T\theta_{Te1}$, where $\theta_{Te1}=N_{Te1}/N_m$ is the coverage of binding sites by target molecules when only they bind. In the case shown in Picture 2c the interferer binding also affects the sensor's transient response, and the sensor's response time is prolonged.

The presented results show that interferer binding can influence the transient and equilibrium response of the affinity-based sensors in various ways, depending on parameters of both target and interferer molecules, their concentrations and their affinity properties. The influence on both the equilibrium value and the response time of the sensor can be significant. Therefore, the interferer binding can cause false alarms and other misinterpretations of measured data (e.g. wrong identification or incorrectly determined agent concentration). This points to the need for interference binding to be minimized by using adequate techniques in order to improve the selectivity and effectiveness of chemical and biological sensors. Since this is not always possible, the interferer binding must be taken into account in the analysis of the output signal of sensors used for detection of target agents occurring in small concentrations in the environment, together with a multitude of other more abundant substances. The presented analysis is also useful for development of methods for simultaneous detection of multiple agents, as well as of pattern recognition methods based on sensor arrays.

4. CONCLUSION

In both civilian and military applications it is extremely important to correctly identify a chemical or biological agent and to accurately determine its concentration. In this paper the problem of limited selectivity of MEMS/NEMS affinity-based sensors for detection of chemical and biological agents is considered. The results show that binding of a non-target substance can cause a significant change in binding kinetics, i.e. in both the equilibrium value and the rate of the sensor's response. The presented analysis is useful to provide the guidelines for improvement of both accuracy and effectiveness of detection of the target substance concentration in methods based on time domain measurements of the sensor's output signal. It is also useful during the development of methods for simultaneous detection of multiple substances.

ACKNOWLEDGMENTS

This work has been partially funded by the Serbian Ministry of Education and Science within the project TR32008.

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