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Development of an enzyme coated microcantilever-based biosensor for specific detection of short–chain alcohols †

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Abstract: This paper describes the development of a biosensor designed to enzymatic detection of short-chain alcohols. The biorecognition element, alcohol dehydrogenase, was immobilized on self-assembled monolayers deposited on top of silicon nitride microcantilevers. The self-assembly process was performed by surface activation using 3-aminopropyltriethoxysilane, followed by glutaraldehyde and biomolecule binding. X-ray photoelectron spectroscopy and atomic force microscopy were used. The biosensor showed a lower response time, a sensibility from 0.03 to 1.2 mL/L. Its selectivity was analyzed through exposure to pure and mixed volatile solvents. Sensor sensibility was higher in the presence of short-chain alcohols family and practically null involving others polar or nonpolar solvents.

Keywords: biosensor; microcantilever; alcohol dehydrogenase

1. Introduction

Alcohols are important compounds in medicine, biotechnology and mainly in food industry, in which some procedures may involve fermentation and distillation. However, in some cases, the volatilized concentration of alcohols can reach toxic levels, causing inflammation of the nasal and conjunctiva mucous membrane, skin irritation, poisoning besides being highly flammable. Given these circumstances, monitoring the volatilized alcohol concentration in the air is important [1,2]. In nature, the detection of methanol can signal plant immunity, with potential application in plant phenotyping [3].

Detection of small quantities of VOC in gaseous medium requires a sensitive sensor. In this context, the development of microcantilever (μC)-based biosensors has been an efficient solution [4,5]. Microcantilevers are mechanical probes with a special format used to measure small forces and different probes are employed for investigations with atomic force microscopes (AFM) [6].

Initial applications of a microcantilever as a sensor was a mass-sensitive balance, which acted as a microresonator, reaching resolutions in the order of picograms and allowing the detection of individual virus particles [7]. Microcantilevers with high Q factor–in the order of 10,000–and high frequency operations–around 1.5 MHz–allow a resolution of theoretical mass of about 20 ag/Hz [8]. Usually, sensors translate the change in a physical property into measurable electrical signals;

however, in this study, the mechanical response of a μC was examined through AFM. The immobilization of biomolecules, such as enzymes, in a sensor promotes the affinity and high selectivity of catalytically active proteins high selectivity, to detection of a specific target and studies applying alcohol dehydrogenase enzyme immobilization have been reported for detection of alcohols using amperometric [1,9,10] and voltammetric sensors [11,12]. The enzymatic biosensors offer a combination of performance and analytical features not available in other bioanalytical system [13]. Microcantilevers can be coated on either sides or just one side with a biosensitive layer in a process called functionalization, making it possible to detect the mass variation in the set by changes in the resonant frequency. This variation of the technique is commonly used in liquid medium [14].

1.1. Microcantilever Surface Activation

The chemical modification or activation of microcantilever surfaces for the attachment of biomolecules is commonly performed using reagents such as 3-aminopropyltriethoxysilane (APTES) and alkanethiols, such as 11-amino-1-undecanethiol hydrochloride (THIOL) [15–17].

1.2. Immobilization of Biomolecules on Microcantilever Surfaces

Immobilization of biomolecules on microcantilever surfaces can be seen as closely related to the immobilization methods used to fabricate electrodes; these procedures are gathered under the generic term "chemically modified devices" [15].

In this paper, was present a specific enzymatic biosensor that uses a signal transduction based on mechanical displacement, which differs from commonly commercially available voltammetric and resistive sensors.

2. Materials and Methods

2.1. Reagents

All chemicals and buffer components were used as received. The solvent was provided by J.T. Baker and other products as APTES, triethylamine and glutaraldehyde (GLD) were used as received from Sigma Aldrich.

2.2. Microcantilevers (μCs)

Silicon nitride microcantilevers used were HA_NC model (NT-MDT) with stems at both ends, being (A) the shortest and (B) the longest, as Table 1 shows.

Table 1. Physical characteristics of the microcantilevers used in this study.

Characteristic	A	B	Typical dispersion
Length, L (μm)	94	124	± 2
Width, W (μm)	34	34	± 3
Thickness, H (μm)	1.85	1.85	± 0.15
Force constant (N/m)	12	3.5	± 20%
Resonant frequency (kHz)	235	140	± 10%

2.2.1. Microcantilever Functionalization

2.2.1.1. Surface Activation

Microcantilevers were subjected to a heat treatment at 500 °C for eight hours, subsequently washed with piranha solution and then extensively washed with milli-Q water to remove the excess of this solution. After this process, the microcantilevers were submitted to an activation procedure through vaporization of 40 μL of APTES and 40 μL of triethylamine in a nitrogen atmosphere for one hour [16].

2.2.1.2. Biomolecule binding

The functionalization through the formation of to the self-assembled monolayer (SAM) activated with APTES [16,18,19] was performed with alcohol dehydrogenase enzyme stock solution (0.25 mg/mL) dissolved in 50 mM sodium phosphate buffer, pH 8.6.

2.3. Instrumentation (Scanning Probe Microscopy (AFM))

The frequency response of the microcantilevers during excitation was measured using a Veeco Dimension V AFM.

2.4. X-Ray Photoelectron Spectroscopy (XPS)

The XPS spectra were acquired to identifying and quantifying all the chemical elements on the surface of the sample (μC), using a spectrometer from Scienta Omicron.

3. Results and Discussion

In the Figure 1a a comparison between the response of a bare (Control) and functionalized μC when are exposed to vapor of 10 μL of ethanol and is showing the short response time of the biosensor less than 1 s (A), in (B) is showing the influence of the surface tension, depicts adsorption, as well as a total recovery of the bioactive layer after 10 min (C). The Figure 1c is showing the sensibility from 0.03 to 1.2 mL/L. The reproducibility of the measurement (Figure 1b), was carry out 3 times, using the same experimental condition of the Figure 1a.

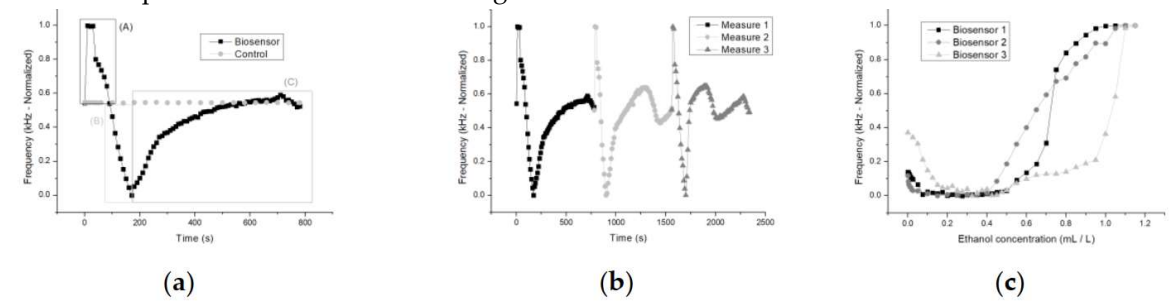

Figure 1. (a) Comparison of the response of a bare (Control) (●) and a functionalized (Biosensor) (■) μC exposed to 10 μL of ethanol vapor; (b) Three different biosensors with steam powered APTES; (c) Resonant frequency variation as a function of ethanol concentration from 0 to 1.2 mL/L.

Its selectivity was analyzed through exposure to pure and mixed volatile solvents (Figure 2). Sensor sensibility was higher in the presence of short-chain alcohols family (methanol, ethanol and propanol) ranging from 0.45 to 0.85 kHz and practically null involving others polar or nonpolar solvents.

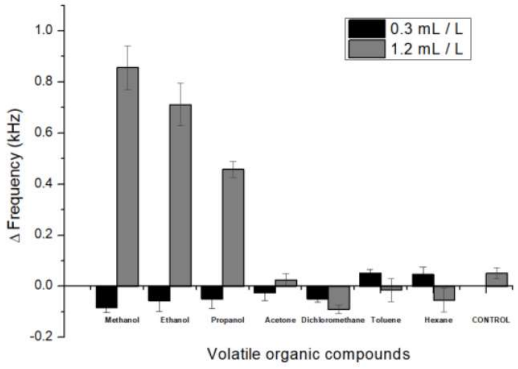

Figure 2. Selectivity test with different VOCs at concentration range from 0 to 0.3 mL/L (black bars) and 0.4 to 1.2 mL/L (gray bars).

4. Conclusion

The functionalization of microcantilevers with alcohol dehydrogenase enzyme immobilized in self-assembled monolayers allowed the construction of a biosensor for selective detection of short-chain alcohols, at ambient conditions, even in the presence of a mixture of VOCs. The biosensor was evaluated using AFM, with dynamic mode and contact mode to obtain 3D image of the surface, as well XPS to identifying and quantifying all the chemical elements on the surface of the μC. The biosensor developed showed less susceptible to humidity and the temperature variations, presenting a high-quality factor, a faster response time, selectivity, sensitivity, and durability.

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