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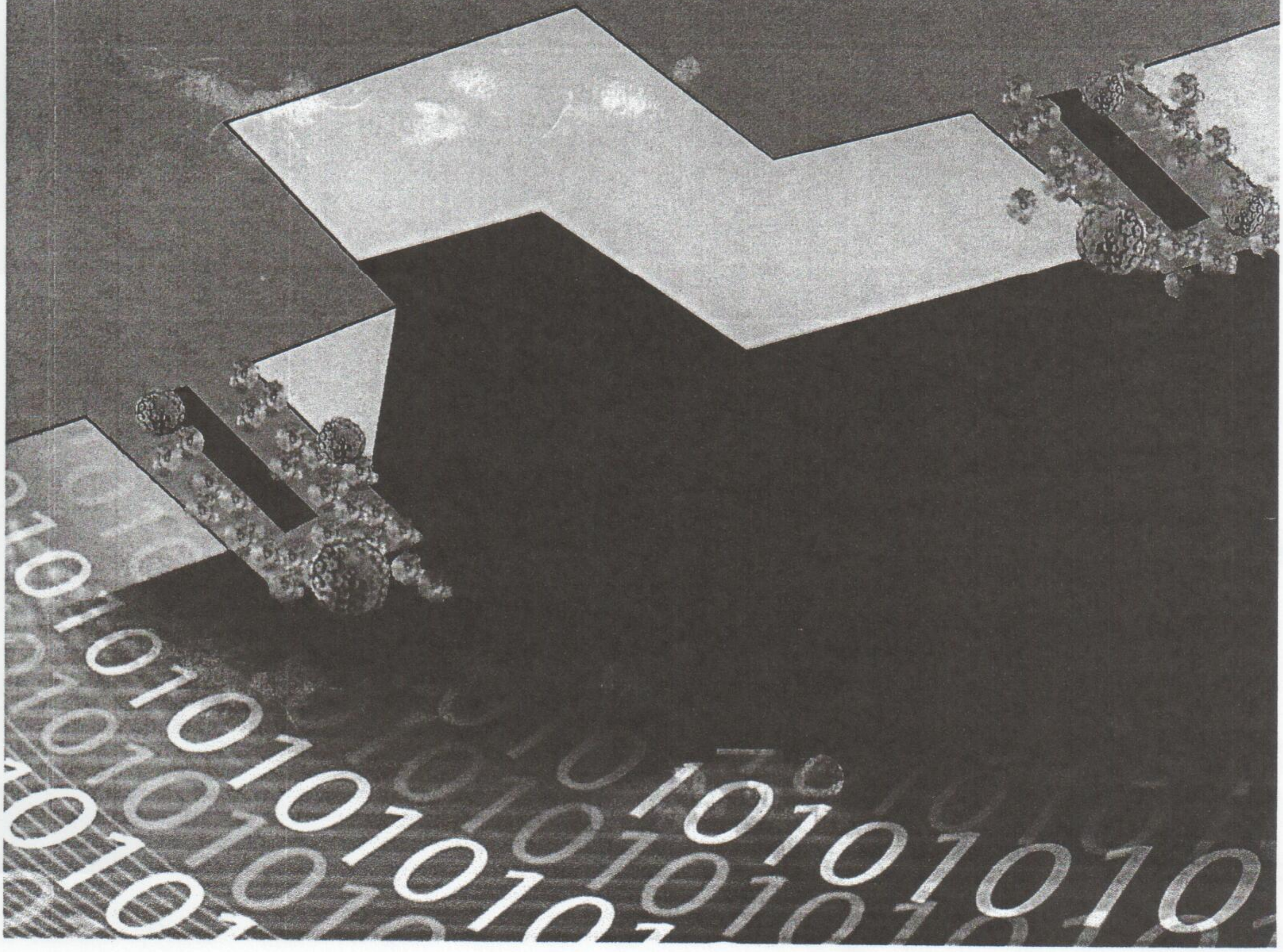

Microcantilever biosensors containing immobilized alkaline phosphatase enzyme for detecting lead in water*

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Abstract – **It was prepared a biosensor for detecting lead using a MC sensor containing immobilized alkaline phosphatase enzyme on the sensor surface. It was evaluate the sensitivity and detection limit of MC-B to different concentrations of lead ions (0.1 a 100 μg mL^{-1}) with resonance frequencies measure using an AFM (Dimension V (Veeco)). The results indicated a change in tridimensional conformation of the enzyme with the binding of divalent metal that lead to smoothest surface. The sensitivity and limit of detection for MC-B were 35 ng/ppm and 0.18 x 10^{-6} molL^{-1}, respectively.**

Index Terms – ***microcantilever, biosensor, alkaline phosphatase, AFM.***

I. Introduction

The great interest in developing microcantilever-based biosensors (MC-B) is due them can be utilized with biomolecules with high specificity for sensing target analytes, without limiting signal transduction between the biomolecule and an electronic sensing device [1]. The advantages of microcantilever-based biosensors are that they are small, inexpensive, provide label-free detection, and may be used in sensor arrays [2]. The functionalization of microcantilever surface plays the key role for the development of the MC-B. Thus depending upon the final application of these devices different types of immobilization can be used [3]. The purpose of the present investigation was to prepare a biosensor for detecting lead using a MC sensor coated with immobilized alkaline phosphatase enzyme on the surface. The figures 1 (a), (b), (c) and (d) are showing steps for a better comprehension of physical chemical properties of MC-B surface, used in this investigations.

The experiments were performed with commercially available rectangular silicon tip-less microcantilevers (350 μm lengths, 30 μm width and 0.5-1.5 μm thickness with a force constant of 0.07 N m^{-1}, NT-MDT Company, Russia).

The development of the microcantilever-based biosensors was carried out in the following steps (Fig. 1): (a) the microcantilever surface was oxidized with "piranha" solution (1:3 H_2O_2:H_2SO_4); (b) the oxidized surface was functionalized with 5 μL of (3-Mercaptopropyl)-trimethoxysilane (silane) by dip-coating technique and (c) 10 μL (5 mg in 1 mL) of alkaline phosphatase enzyme was deposited on the silane surface by dip-coating technique. It was evaluate the sensitivity and detection limit of MC-B to different concentrations of lead ions (0.1 a 100 μg mL^{-1}) with resonance frequencies measure using an AFM (Dimension V (Veeco)).

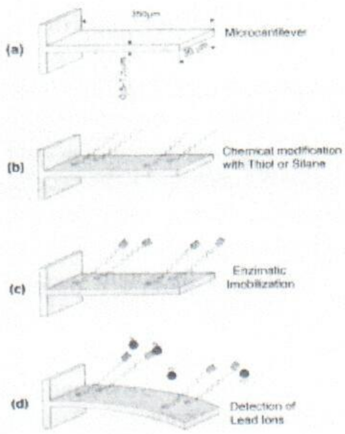

Fig. 1. Scheme of the development of the MC-B used in this experiment.

Physical-chemical properties of MC-B surface were investigated in each steps of development of MC-B by contact angle and AFM techniques. The hidrophobicity and hidrophilicity of each step of the development of the MC-B was determined by contact angle with water drop (Milli-Q®, surface tension of 72.7 mJ/m^2), using a contact angle meter (KSV Instruments). The measurements were performed in triplicate, at 25 °C and 45 % of humidity, depositing around 4.0 μl of water with a Hamilton syringe. Surface morphology and average roughness of each step of development of the MC-B were characterized with AFM, using a pyramidal silicon nitride tip attached to a cantilever with a spring constant of 0.03 N/m, in the tapping mode over a 10μm x 10μm scan area. The results of the contact angle (Fig. 2) showed that oxidation of microcantilever surface become the surface more hydrophilic, due OH ions. This hydrophilicity of surface improved the efficiency of functionalization with silane. Then after silane deposition, the average contact angle in biosensors surface increased (surface more hydrophobic).

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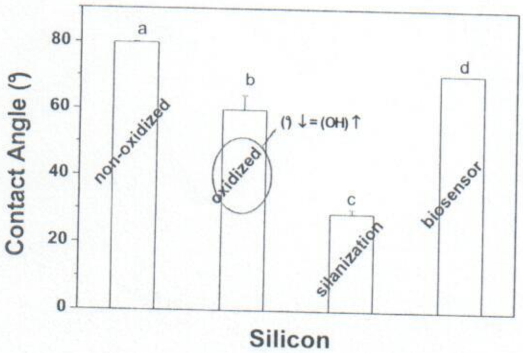

Fig. 2. Contact angle of each step of the development of the MC-B: (a) microcantilever surface without oxidation, (b) microcantilever surface oxidized with "piranha" solution, (c) oxidized surface of microcantilever functionalized with silane and (d) deposition of alkaline phosphatase enzyme on silnae surface.

Fig. 3 shows the 3D images of the steps of construction of the MC-B. The roughness of these images were obtained using the software for data analysis Gwydion © 2.1. It was observed that the surface roughness increased with the immobilized enzyme on the silane. This roughness in biosensor surface was reduced by binding with divalent metals (Pb^{+2}). This fact can be an indicative of a change in tridimensional conformation of the enzyme with the binding of divalent metal that led to smoothest surface.

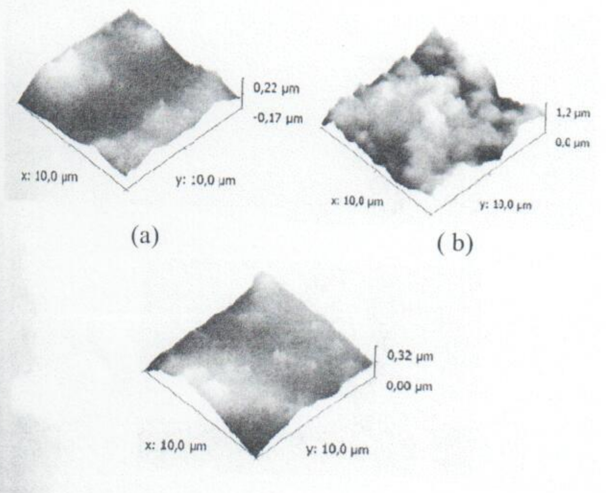

Fig. 3. 3D images obtained by AFM to each step of construction of the MC-B: (a) silane, (b) alkaline phosphatase enzyme and (c) Pb^{+2}.

The sensitivity and limit of detection of biosensor proposed were determined from the response of the deflection of microcantilever-based biosensors (MC-B) (nm) to different concentrations of lead showed in the figure 4. The temperature and humidity in the room were monitored every twenty minutes by a thermohygrometer (MINIPA MT-241).

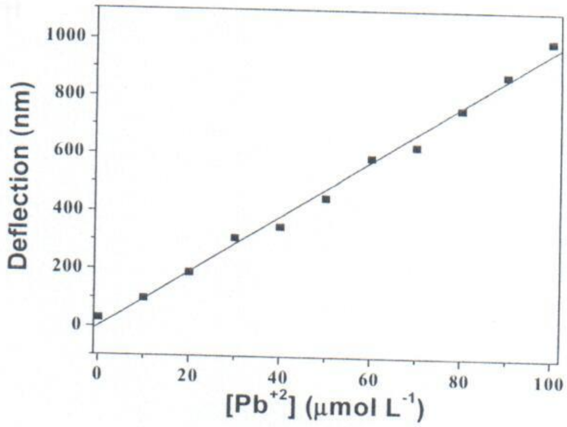

Fig. 4. Deflection (nm) *versus* different concentrations of lead. (—) linear regression.

It was verified that the curve of the deflection *versus* different concentrations of lead exhibited a good linearity with a regression coefficient of $R^2 > 0.97$. The sensitivity and limit of detection for the MC-B functionalized with silane were 35 ng/ppm and 0.18×10^{-6} mol/ L, respectively. It was observed a stable and repeatable response of deflection (nm) *versus* different concentrations of lead. The excellent sensitivity shown by the proposed microcantilever sensor can be related to alkaline phosphatase enzyme layer, which is able to bind at lead. When a phosphate buffer pH 7 was used for rinsing of MC-B, the lead molecule was easily removed, indicating the good reversibility of the sensor proposed. This rinsing did not remove the enzyme layer, demonstrating that it was adequately linked to the silane layer.

Conclusion

The results indicate that the biosensor constructed from micromechanical microcantilevers used in atomic force microscopy has promising properties of transduction in the sensing field of heavy metals, applied to evaluate the water quality .

Acknowledgment

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