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Activities abstracts

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Ministério da Ciência e Tecnologia — Governo Federal BRASIL País rico é país sem pobreza — CAPES — CNPq — FAPESP — Centro de Tecnologia da Informação Renato Archer

A 4-3.4 SURFACE FUNCTIONALIZATION OF MICROCANTILEVERS WITH BIOMOLECULES

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I. INTRODUCTION

The ability of microcantilever, used in atomic force microscopy, to bend (deflection) as a function of molecular interactions at the cantilever surface or to vary the specific resonance frequency due to mass loading, allows its use as an excellent transducer to physical, chemical and biological sensors. Recent advances in the design and development of these sensors provide a simple microelectromechanical system, easily microfabricated and potentially have very high sensitivity [1].

Cantilever biosensors have attracted considerable interest in several areas because of the highly specificity of certain biomolecules (antibodies, enzymes, DNA, etc.) and can detect multiple biomarkers simultaneously with high sensitivity and selectivity using very small volumes of sample. According to the IUPAC definition [2], a biosensor is a sensor which has biological components (e.g. cells, organelles, or macro molecules) and detects their interaction with the analyte by using a dedicated transduction mechanism. Potential applications of these cantilever biosensors include analysis in the fields like biomedical, environmental and agricultural [3]. Other applications of interest in agribusiness are the monitoring of pesticides and metals in water. Regarding the determination of heavy metals in water, it is reported the immobilization of the enzyme (alkaline phosphatase) in various matrices for the development of biosensors for detection of metals such as cadmium, cobalt, zinc, nickel and lead in water [4]. This paper proposes the development of devices for heavy metal analysis using alkaline phosphatase immobilized on the microcantilever surface.

II. ACTION AND RESULTS

Figure 1 shows the characteristics of microcantilever and the construction steps of device.

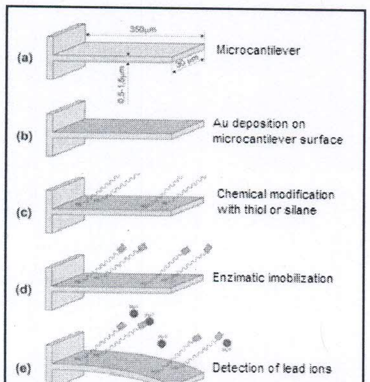

Figure 1 – a) microcantilever characteristics and (b, c, d, e) construction steps of device.

The zeta potential of alkaline phosphatase was determined by electrophoretic mobility at pH between 2.5 and 10.5, using a spectrometer Malvern Nano-ZS (Malvern Instruments, UK). The resonance frequency of cantilever was monitored with Dimension V (Veeco) atomic force microscope.

The isoelectric point obtained in Figure 2 for the solutions used in the immobilization of alkaline phosphatase is 3.5. This pH value is showing that the immobilization have a predominance of protonated carboxylic groups. Thus, the enzyme was immobilized on the cantilever's surface owing to the fact that the interaction of the carboxylic group present in the thiol (16-mercaptohexadecanoic acid) and the few amine groups that were protonated. This fact indicates that the enzyme was weakly immobilized on gold surface, allowing a great mobility of this hydrolase on the immobilization matrix.

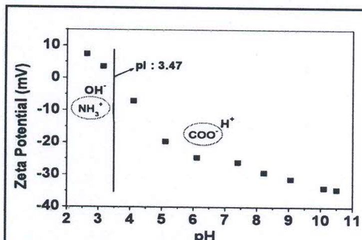

Figure 2 – Influence of pH on the zeta potential of the solution of alkaline phosphatase.

The microcantilever surface was characterized with AFM. Table I contains the roughness of the construction stages of the device obtained with the use of Gwyddion © 2.1 software.

Table I shows an increase in roughness with the enzyme immobilization on the gold surface modified with thiol and a decrease with the binding of divalent metals (Pb+2) in the enzyme. This result indicates that the enzyme binds weakly to the gold, changing its conformation with the binding of divalent metal to a conformation that makes the surface smoother.

Table I. Roughness of films on the microcantilever in the construction stages of device.

Construction Stages	Ra (nm)	Rms (nm)
Thiol	80.8	101
Enzyme	194	240
Pb^{+2}	34.0	44.6

III. REFERENCES

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