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Journal Item

How to cite:

Psoma, Sotiria D.; van der Wal, Peter D. and de Rooij, Nico F. (2011). Low Fluorescence Enzyme Matrices Based on Microfabricated SU-8 Films for a Phenol Micro-Biosensor Application. *Procedia Engineering*, 25 pp. 1369–1372.

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Version: Version of Record

Link(s) to article on publisher's website:

<http://dx.doi.org/doi:10.1016/j.proeng.2011.12.338>

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Proc. Eurosensors XXV, September 4-7, 2011, Athens, Greece

Low Fluorescence Enzyme Matrices Based on Microfabricated SU-8 Films for a Phenol Micro-Biosensor Application

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Abstract

In this contribution, the possibility of using SU-8 photoresist, a polymer widely used in MEMS applications, for the development of inexpensive and disposable optical phenol micro-biosensors is explored. The immobilisation of the enzyme, the encapsulation of the indicator and the patterning of the SU-8 were accomplished simultaneously in a simple one step microfabrication process. The enzyme still showed activity after encapsulation in SU-8 although the process involved its embedding in a hard and rigid epoxy resin matrix. This was carried out by measuring the signal of an oxygen-sensitive indicator (ruthenium-complex) through monitoring of the enzymatic oxidation of phenol which consumes oxygen. Films without enzyme showed negligible variation in fluorescence intensity upon phenol addition, whereas films with encapsulated enzyme and oxygen-sensitive fluorescent indicators showed a very clear change in fluorescence intensity upon addition of phenol. The current work demonstrates a new concept of a low cost immobilisation technique in combination with the microfabrication process for biosensor technology.

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Keywords: SU-8 films; microfabrication; immobilisation; tyrosinase; phenol biosensor; fluorescence.

1. Introduction

Phenol is one of the most important and widely used industrial chemicals and studies have shown the existence of phenols as pollutants of air, water and soil. The determination of phenols is of particular

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interest due to their widespread usage by industry and because of their toxic effects upon aquatic organisms and persistency in the environment [1]. Many different analytical methods including gas chromatography, high performance liquid chromatography, colorimetry, capillary electrophoresis, utilisation of nanoparticles and a large number of electrochemical biosensors based on tyrosinase and peroxidase have been developed for the detection of phenolic compounds in biological and environmental samples [2]. All methods for laboratory analysis of phenols are non-specific. This lack of sensitivity necessitates a great deal of sophistication and quite expensive instrumentation [3].

Biosensors based on tyrosinase are simple and convenient tools for phenol assays due to their high sensitivity, simplicity and effectiveness. Tyrosinase is a copper-containing monooxygenase enzyme that catalyses the conversion of phenolic substrates to catechol, which is then oxidized to quinone. Amperometric detection has been widely used in most tyrosinase-based phenol biosensors, because quinone can be electrochemically reduced to allow convenient low potential detection of phenolic compounds. However, optical biosensors based on absorbance and fluorescence detection of phenol present several advantages over amperometric detection. Only three optical biosensors based on immobilized tyrosinase were presented so far in the literature, differing on the supporting membrane; viz nylon membrane, chitosan and polyelectrolyte multilayers with a cationic polymer [4]. In the two first cases the optical detection was based on absorbance changes and required the addition of an exogenous compound, and in the third both absorbance and fluorescence changes were measured.

The development of an optical micro-biosensor for monitoring phenols in drinking and waste water is presented based on fluorescence spectroscopy in combination with a specific microfabrication process. In order to achieve this goal the interaction between a biological component (the enzyme tyrosinase) and the specific organic pollutant (phenol) were monitored using an oxygen-sensitive fluorescent indicator. In addition the utilisation of the widely used SU-8 photoresist as an immobilisation matrix for tyrosinase for the development of phenol micro-biosensor is presented.

2. Experimental Work

The straightforward utilisation of the commercially available SU-8 formulations was not an appropriate and satisfactory route and the “customized” SU-8 resist was prepared by dissolving the Epon SU-8 granules in gamma-butyrolactone (GBL), and adding the photoinitiator triarylsulfonium hexafluoroantimonate salts. Detailed preparation of the “customized” SU-8 solutions and films has been described in earlier publications [5] and [6], with some adjustments to the new application. In all experiments, the composition of SU-8 solutions was 40% SU-8 granules and 60% solvent (40/60) with 2.5% photoinitiator. Silicon wafers were cleaned and dehydrated according to standard clean-room protocol and were covered with a sacrificial layer of dextran by spin-coating a 5% w/v solution in distilled water on its surface. This sacrificial layer can be dissolved in water for easy release of the SU-8 structures (rectangular platelets with dimensions of 5mmx4mm). The standard microfabrication process steps for SU-8 resist with or without enzyme and oxygen sensitive fluorescent indicator formulations can be found in reference [6]. After processing, the resulted SU-8 films were highly transparent without any evidence of cracks with an approximate thickness of 5.4µm. Finally, the structures were released in distilled water, collected and stored in 10mM phosphate buffer solution at a temperature of 5°C. SEM images with surface topography of the resulted SU-8 films can be observed in Figures 1(a) and 1(b).

3. Results and Discussions

Fluorescence spectroscopy experiments were carried out using the SU-8 film matrices in order to assess their suitability as sensing elements. The principle of the proposed sensor is based on the

enzymatic oxidation of phenol and the measurement of the oxygen consumption using an oxygen sensitive fluorescent indicator. A high transparency of the SU-8 films was observed, which is a significant advantage for an optical detection method. The method used to entrap the oxygen indicator inside the polymer matrix involved the utilisation of the tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) dichloride oxygen sensitive fluorescent indicator included in the initial SU-8 solution and the standard microfabrication process carried out. The concentration of the oxygen indicator was 10mM in the GBL (gamma butyrolactone) organic solvent, which was subsequently added in a quantity of 100µm to the initial SU-8 solution (4ml). The results from the experimental work using a fluorescence spectrophotometer showed that only the second method proved to be sensitive to phenol concentration changes during the oxidation reaction whereas the presence of microspheres did not produce any tangible variation in fluorescence intensity other than excessively noisy signals.

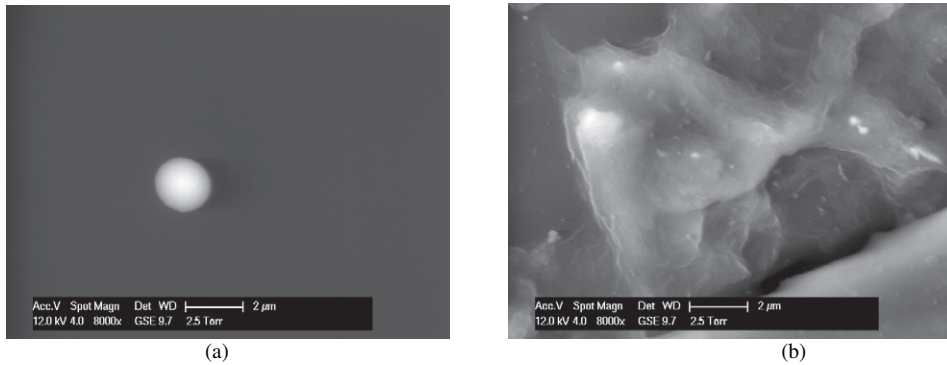


Fig. 1. (a) SEM pictures of SU-8 plain films; (b) and, Su-8 films with immobilised tyrosinase and oxygen indicator.

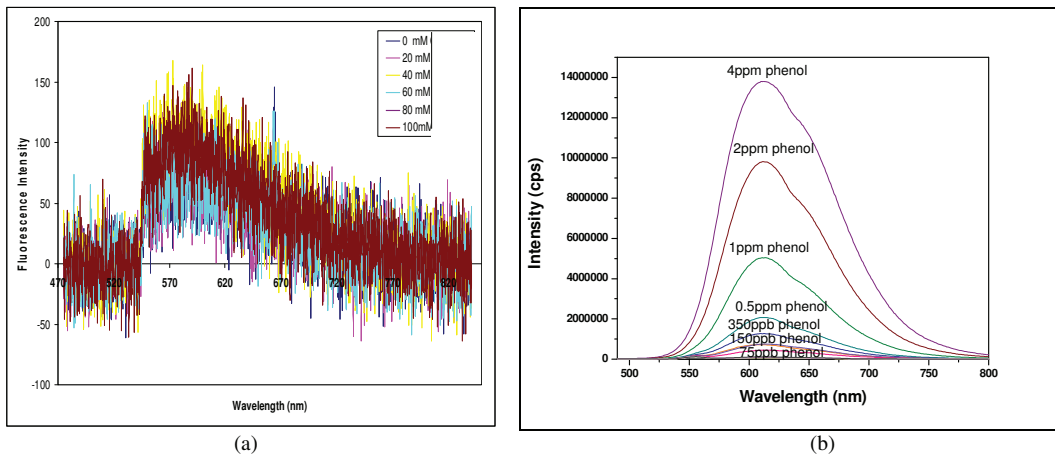


Fig. 2. (a) Fluorescence spectra using only SU-8 films; (b) fluorescence spectra using Su-8 films with immobilised tyrosinase and oxygen sensitive fluorescent indicator.

In Figure 2(a), the fluorescence spectra of SU-8 films with different added phenol concentrations are presented. In these experiments, a number of 3 standard SU-8 films with encapsulated enzyme were placed in a 1ml quartz cuvette containing 10mM phosphate buffer. The enzyme quantity was added in the 4ml SU-8 solution before the microfabrication process was equal to 0.5mg of tyrosinase (4,276 units/mg solid) diluted in phosphate buffer 10mM, pH7.4. No oxygen indicator was added during the preparation

of the microfabricated SU-8 films that were used in the present experiment. Although the phenol oxidation takes place, there is no fluorescence detected due to the absence of any oxygen sensitive fluorescent indicator. The effect of the addition of the oxygen indicator is shown in Figure 2(b) where a clear difference among the spectra can be observed with different phenol concentration. As the reaction takes place, the consumption of available oxygen during the reaction is proportional to the added phenol concentration and hence it causes a reduction in fluorescence intensity of the oxygen sensitive indicator.

Another set of experiments was performed and fluorescence spectra were obtained from samples including SU-8 films of 5 variable thickness. The effect of thickness appears to be very important as it is inversely proportional to the level of fluorescence intensity measured; the thinner the film used, the higher the fluorescence intensity measured. This can be attributed to the enzymatic activity caused by the enzyme molecules which are entrapped inside the volume of the film and not only by the ones that are located on its outer surfaces.

4. Conclusions

From the results presented above, a general conclusion is that the optical measurements proved that the enzyme remains active inside the transparent SU-8 films and the proposed microfabrication process did not damage it. In addition, the oxygen sensitive fluorescent indicator did not degrade during the microfabrication process and is present inside the SU-8 microfilms. One of the advantages using SU-8 is that the plain SU-8 films showed negligible fluorescence as well as the films with the encapsulated enzyme only. The strong advantage of the proposed approach is the simultaneous enzyme and indicator entrapment during the microfabrication process within a single step, which is of high importance for the simplification of BioMEMS procedures; and, it is valid proposal for the construction of disposable, low-cost and fast micro-biosensors for phenol detection.

Acknowledgements

Part of this work was financially sponsored by Swiss National Science Foundation (Project No: IZK0Z2-124260). The authors thank the staff of Division C at CSEM-Neuchatel for their lab support.

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