The small molecule nitric oxide (NO) is known to mediate many physiological processes. In this study we married the silicon planar technology with modified electrodes, to explore the electrochemical detection of that molecule by amperometric measurements. Typical dimensions of the sensor are: a length of 10 mm and a width of 3 mm. It contains 8 gold working electrodes (10 $\mu$m x 10 $\mu$m), a Ag/AgCl reference electrode and a gold counter electrode. The activity of nitric oxide and nitrite was studied in the micromolar range with and without covering the electrodes with a thin film of Nafion®. Nitric oxide and nitrite presented a oxidation peak around 760 mV and 820 mV, respectively. It was observed that Nafion® decreased the nitrite activity by a factor of 8. It was also observed that the nitric oxide sensitivity is higher than nitrite and better response for nitrite at low concentrations was obtained, indicating the importance of using Nafion® in the detection of nitric oxide at low concentrations to avoid NO$^{-2}$ interference.

**KEYWORDS**

Microelectrodes, nitric oxide, modified electrodes, amperometric detection.

**INTRODUCTION**

Microelectrode arrays based on silicon technology have been used with success on several applications, such as action potentials and single neuron activity detection$^1$. Due to the small dimensions involved, microfabrication presents many advantages including: batch fabrication, easy shape definition by computer designed masks, high reproducibility and low cost$^2$. In this study we married the technology of silicon planar processing with modified electrodes to explore the electrochemical detection of the nitric oxide (NO) molecule using amperometric measurements$^3$. 
The NO molecule have attracted attention in the recent years because of its biological activity in the mammals, including humans\textsuperscript{4}. NO measurement in biological systems is difficult due to the small amounts present, usually in the nanomolar range, and its rapid reaction with oxygen generating nitrite (NO\textsubscript{2}\textsuperscript{-}). The detection of the activity of such molecules in small concentrations is vital in many studies \textit{in vitro} and \textit{in vivo}, as well as in diagnostic and therapy\textsuperscript{5}. We have developed a general purpose silicon based electrochemical sensor composed of an array of microelectrodes. The differential pulse voltammetry (DPV) technique was used to detect the activity of both NO and NO\textsubscript{2} in solution with and without covering the electrodes with Nafion\textsuperscript{®}.- a well known cation exchanger.

SENSOR DESIGN AND FABRICATION

The sensor is composed of a silver/silver chloride reference electrode, a gold counter electrode (2 mm x 0.5 mm) and 8 gold working electrodes (10 µm x 10 µm). Typical dimensions of the sensor are: a length of 10 mm and a width of 3 mm. Silicon nitrite film was used as a protective material. Details on the sensor design and fabrication is described in a previous work\textsuperscript{6}. Figure 1 shows SEM images of the fabricated sensor where the contact pads and the electrodes can be seen. It is also shown the sensor fabrication sequence.

![Figure 1 - SEM image of the sensor front side and the fabrication sequence.](image-url)
EXPERIMENTAL PROCEDURE

We prepared 50 mM of purged (N₂) phosphate buffered solution (PBS; pH = 7.4). The nitric oxide solution was obtained by bubbling NO gas (10 % in N₂) during 1 hour in a gas tight burette. The micromolar concentrations was achieved by adding microliters of the NO saturated solution in the cell. For the nitrite solution, continuous additions of 75 µl of 10 mM sodium nitrite (NaNO₂) was done. In order to cover the electrodes with Nafion® (1% in ethanol), one drop of it was applied and left to dry, in order to get an uniform coverage. Nafion® was used in all NO detections. After each addition of the analyte the solution was stirred. All experiments were performed with a fully computer controlled three-electrode potentiostatic system (BAS - Bioanalytical System CV 50W), in a Faraday cage. The DPV conditions were: potential range (400 mV to 900 mV), potential sweep rate (20 mV/s), pulse amplitude and width (50 mV), pulse period (200 ms) and sample width (17 ms).

RESULTS AND DISCUSSION

It is shown in figure 2a results for 50 µM of nitric oxide, and nitrite with and without Nafion®. It can be seen that NO and NO₂ oxidation occur around 760 mV and 820 mV respectively. It is clear the role played of the Nafion® film, reducing the nitrite peak by a factor of 8.

![Fig.2](image)

Fig.2 - Results for: a) 50 mM of NO (-o-) and NO₂ with (dash) and without (-dash-) Nafion® and b) current x concentration for NO (-o-) and NO₂ without Nafion® (-) in the micromolar range.
Figure 2b shows the current x concentration curve for NO and NO$_2$ (without Nafion®) in the micromolar range. It is observed that the nitric oxide sensitivity (slope of the curve) is higher than nitrite although better response for nitrite at low concentrations was obtained. This results indicates the importance of using Nafion® in the detection of nitric oxide at low concentrations levels.

CONCLUSIONS

This paper described the design and fabrication of an array of gold microelectrodes based on silicon planar technology, composing a general purpose electrochemical sensor. The activity of nitric oxide and nitrite was studied in the micromolar range with and without covering the electrodes with a Nafion® film. Nitric oxide and nitrite presented a oxidation peak around 760 mV and 820 mV, respectively. It was observed that Nafion® decreased the nitrite activity by a factor of 8. It was also observed that the nitric oxide sensitivity is higher than nitrite and better response for nitrite at low concentrations was obtained. Our results suggests the importance of using Nafion® in the detection of nitric oxide at low concentrations to avoid NO$_2$ interference. Now, studies involving new catalytic films joined with FIA technique are in course to detect NO at nanomolar concentration.

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