Solid State Nitric Oxide Sensor Using a Latex Rubber Matrix

R. D. Herculano, C. A. Brunello, C. F. O. Graeff*

Summary: In this work we propose a sensor made of a spin trap (iron(II)-diethyldithiocarbamate complex, FeDETC) encapsulated in a latex rubber matrix. Latex was used as solid matrix due to its excellent mechanical properties, biocompatibility and natural angiogenic properties. The fabrication and optimization processes were done by varying: $FeCl_3 6H_2O$ and DETC concentrations in the system, polymerization temperature, FeDETC/latex ratio. The sensor presents high stability, the observed Electron Paramagnetic Resonance signal can be detected even after the sensor is exposed to ambient atmosphere for 40 days. It can be reused and is able to measure NO concentration as low as 0,1 mM. These results make NO-FeDETC in the rubber matrix a promising NO sensor.

Keywords: FeDETC; latex matrix; nitric oxide sensor

Introduction

Nitric oxide (NO) has been found to play an important role as a signaling molecule in many parts of the organism as well as a cytotoxic molecule of the nonspecific immune response. NO is a small neutral free radical, which makes it very reactive and unstable. In air, it quickly reacts with oxygen to forming NO_2.[1] NO exists in interstellar space[2] and is present in the atmosphere of Venus and Mars.[3] Since the discovery of it's role in endothelial muscular relaxation in 1987 a great effort has been made in order to find methods for its detection and quantification *in vitro*, *in vivo* and *ex vivo*.[4] The detection of NO presents a series of challenges since it is highly reactive, which means it has short lifetimes in living organisms, depending on the environment from ms to minutes, before it reacts and consequently is found in low quantities in biological systems. Several methods for NO detection have been developed and one of the most powerful methods for selectively measure NO production in biological systems is Electron Paramagnetic Resonance (EPR) spin trapping techniques.[5] Iron complexes with dithiocarbamates and porphyrins are used as spin traps due to the affinity between NO and the iron complexes, however these complexes are unstable for long-term measurements. Of all dithiocarbamates one of the most commonly used is the hydrophobic iron-diethyldithiocarbamate (FeDETC)[6] both *in vivo* and *in vitro*.

In a previous work we have shown that a NO sensor based on the entrapment of FeDETC in a silica matrix[7] offers numerous advantages.[8] Solid sensors are easier to manipulate, allow species detection with less contamination with respect to liquid solutions containing spin traps. They can be used for continuous sensing and are stable. For this particular silica based sensor, the NO signal could still be detected even after 40 days of the sensor being left in air. However, sol-gel silica is fragile.

In this work we propose a sensor based on the encapsulation of FeDETC in a solid rubber latex matrix. Recent studies revealed that latex can cause natural angiogenesis and this capability can be controlled.[9–11] It

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also shows low rejection in mammals, and is easy to use. These characteristics indicate that latex rubber[12] may be a good choice for a NO sensor matrix, with superior mechanical properties compared with our previous silica sol-gel based sensor.

Experimental Procedures

The preparation of the sensor was done in two stages: i) the preparation of the latex suspension and FeDETC solution, and ii) the process of mixing the solutions to obtain the modified latex membrane. For the FeDETC solution iron(III) chloride hexahydrate ($FeCl_3.\ 6H_2O$), dimethylformamide (DMF, C_3H_7ON), sodium dietyldithiocarbamate (DETC, $C_5H_{10}NNaS_2 \cdot 3H_2O$) purchased from Acrós Organics (Belgium) were used. The solution was prepared using 12 mg of iron chloride and 20 mg of DETC in 3 mL of DMF under magnetic agitation during 10 minutes. The latex suspension used in this work consists of a mixture of a noncontrolled variety of clones extracted from Hevea brasiliensis. After the extraction, the material is centrifuged and the pH is changed to about 10, using an NH_4OH solution to avoid coagulation.

The entrapment of FeDETC in the latex matrix was obtained by mixing the latex suspension with the FeDETC solution inside a small glass dish. Leaving this mixture in air for one day results in an elastic membrane, our sensor.

NO was generated in aqueous solution. $NaNO_2$ 10 mM (250 μl), deionized water (750 μl) and $Na_2S_2O_4$ (145 mg) were mixed in an eppendorf tube of 1.5 ml of capacity. In this condition all nitrite present in the solution is reduced to the NO. The NO concentration in solution was 2.5 mM (saturation condition). In some cases water was added to the solution to decrease the NO concentration. Prior to EPR measurements, the sensor was left for 2 h in the NO solution, to guarantee that NO was homogeneously absorbed through out the membrane.

EPR experiments were made in a computer interfaced Varian E-4 X-band spectrometer at room temperature. For EPR measurements, the sensors were removed from the solution, dried, and inserted in a quartz tube. To maximize signal to noise ratio various spectra were summed up, typically at least 10. To avoid sample repositioning induced errors for the stability measurements, the sample was kept inside the resonant cavity. A reference sample with a known and stable amount of spins was used before each measurement.

Results

The following sensor characteristics were evaluated: signal stability, reproducibility, reusability and sensitivity. In Figure 1 the normalized EPR signal intensity (per sensor mass unit) just after the sensor is taken from the NO solution is plotted as a function of time. The best signals of the EPR spectra were obtained with a concentration of $FeCl_3 \cdot 6H_2O$ of 14,8 mM and of DETC of 29,6 mM. As can be seen, the signal decreases with time until it vanishes. For these measurements the sensor was left immersed in a 2,5 mM NO solution for 2 hours, and then left in air and in the dark. Notice the small decrease in signal intensity even after 426 hours. The sensor is very stable: the signal intensity of NO could be

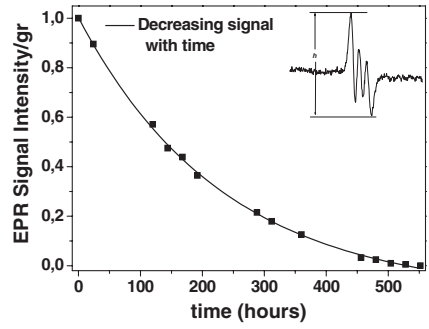

Figure 1.
Normalized EPR signal intensity/gr as a function of time. The insert graph shows a typical EPR spectrum for our sensor.

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detected even after 40 days. To test reproducibility 10 sensors were tested giving similar results in what concerns initial response and signal decay with time.

Figure 2 shows the EPR signal intensity as a function of NO solution concentration. Notice that we can detect NO concentrations as low as 0,1 mM. For this experiment five sensors were used made in identical conditions. They were immersed in solutions with different NO concentrations for 2 hours. It was used concentrations with 0,1 mM, 0,5 mM, 1 mM, 2,0 mM, 2,5 mM. We assume that the molar amount of NO present in each sample is equal to the molar amount of sodium nitrite.

Figure 3 shows the EPR spectra of a fresh and reused sensor. The reutilized sensor consisted of a fresh sensor where the NO signal was monitored for 40 days, like in Fig. 1. This sensor was than inserted in the NO solution, using the same procedure described earlier. As can be seen in Fig. 3, the reused sensor has a reduction in the signal intensity, which can be partially explained by the oxidation of iron in the FeDETC complex from Fe(II) to Fe(III).

Discussion

There are a number of NO sensors in development as well as commercially available.[16,17] As already mentioned solid sensors are easier to manipulate, allow species detection and concentrations measurements with less sample contamination. In comparison with our previous proposal,[7] the present sensor has some improvements. The NO sensor based on the entrapment of FeDETC in silica does retain NO sufficient for EPR detection for about 14 days while that the NO sensor based on latex 40 days. The sol-gel sensor can be re-used however the main problem observed was that the glass tends to break in small pieces, especially after many days left in air. On the other hand, the latex rubber sensor can be re-used without any damage. In what concerns sensitivity, the sol-gel sensor can measure concentrations 10 times smaller than the present sensor. These characteristics indicate that latex rubber may be a good choice for a NO sensor matrix, with superior mechanical properties compared with our previous silica sol-gel based sensor

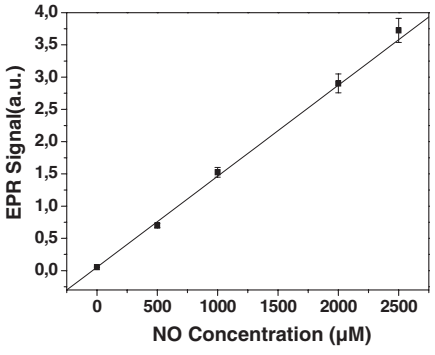

Figure 2.
EPR signal intensity as a function of NO concentration in solution.

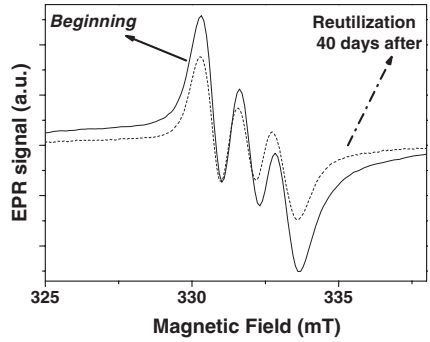

Figure 3.
EPR spectra for a NO sensor: freshly made (solid line), and reused (dash line).

Conclusion

We have shown that FeDETC encapsulated in a latex matrix can be used as a NO sensor. The method of preparation is reproducible and the NO:FeDETC complex is very stable. NO could be detected even after 40 days of the sensor being left in air. The sensor is re-usable with small losses

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in EPR signal intensity. The sensor responds linearly with NO concentration, and can measure down to 0,1 mM (or 100 μM) of NO in water.

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