A new NO sensor based on a Prussian Blue modified electrode.

Martín M$^{1,2}$, Salazar P$^{1,*}$, O’Neill RD$^3$, Roche R$^1$ and González-Mora JL$^1$

$^1$Neurochemistry and Neuroimaging group, Faculty of Medicine, University of La Laguna, Tenerife, Spain.
$^2$Atlántica Biomédica SL, Tenerife, Spain
$^3$UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland.

(*psalazar@ull.edu.es)

Introduction
Nitric oxide (NO) is a major signaling molecule in neurons and in the immune system, either acting within the cell in which it is produced or by penetrating cell membranes to affect adjacent cells. NO has effects on neuronal transmission as well as on synaptic plasticity in the CNS, and there is evidence that NO contributes to the pathology of several diseases including hypertension, Parkinson’s disease and Alzheimer’s disease [1].

The majority of NO detection methods are based on indirect measurements and are hindered by significant drawbacks. For many years now, electrochemical methods have been preferred due to their sensitivity, selectivity and low limits of detection (LOD), adequate to the low range of NO concentration found in the CNS. On the other hand, the possibility of using microelectrodes with high spatial and temporal resolution extends the scope of electrochemical methodology. Electrochemical NO sensors have been mainly developed following two different approaches: (1) based on the direct oxidation of NO on platinum anodes coated with different membranes such as Nafion [2], and (2) based on the electrocatalytic oxidation of NO by a metalloporphyrin film modified electrode [3].

Now we present the possibility of using a new NO sensor based on a Prussian Blue (PB) modified electrode. PB is the most representative compound of the transition metal hexacyanometallate family [4]. Recently, the electrocatalytic oxidation and reduction of NO by PB and its analogues has been demonstrated [4]. Nevertheless, few publications on this topic are found in the literature. The main problem associated with PB modified electrodes is their low stability at physiological pH. In recent reports we presented an optimized method to obtain PB modified sensors with excellent pH stability and selectivity against ascorbic acid (AA) [4].

Methods
Carbon electrodes (screen printed or carbon fiber) were cycled between –0.2 and 1.0 V at a scan rate of 0.1 V s$^{-1}$ (default 15 cycles) in a background electrolyte solution with 1.5 mM FeCl$_3$, 1.5 mM K$_3$[Fe(CN)$_6$] and 2 mM benzethonium choride (BZTC). The background electrolyte consisted of 0.02 M HCl and 0.1 M KCl (pH 1.7). Additionally, an interference-rejection film (Nafion or poly-o-
phenylenediamine (PoPD)) was used. All electro-depositions and calibrations were performed with a standard three-electrode setup: Ag pseudoreference electrode and a platinum wire auxiliary electrode. The PoPD electropolymerization was driven at a constant potential (+0.75 V) for 20–25 min.

To prepare the saturated NO solution, 5 mL of phosphate buffer (pH 7.4) was deoxygenated by purging with pure nitrogen gas for 20 min. This buffer solution was then bubbled with NO gas for 30 min and kept under an atmosphere of NO until use. According to the literature, the NO concentration of the saturated solution is ~1.8 mM [5]. Standard PBS solutions of known NO concentration were prepared from the saturated solution by dilution in deoxygenated PBS.

**Results and Discussion**

During electro-deposition, two well defined peak pairs appeared at ~0.1 and ~0.8 V related to inter-conversion of the different oxidation states of the PB film. The first peak pair corresponds to the inter-conversion of Prussian White (PW, fully reduced form) and PB, and the second pair from PB to Prussian Green/Yellow (PG/PY, fully oxidized form). These reduction and oxidation reactions are supported by free diffusion of cationic and anionic species, respectively (see scheme 1). Until now, we focused our efforts on the use of PB in biosensor design, due to its catalytic activity for H$_2$O$_2$ reduction [4]. Nevertheless, other small molecules such as NO may penetrate the PB lattice structure and undergo catalytic oxidation/reduction (see scheme 1).

The electrocatalytic oxidation of NO on a Prussian Blue (PB) modified electrode, where PY represents the fully oxidized form of PB.

Mechanisms for the two reactions have been suggested for acid media [6], where the vast majority of studies to date have been carried out. Figure 1a shows the evolution of the two characteristic PB peak pairs (PW/PB and PB/PY interconversion) in acidic background electrolyte at different NO concentrations. From these data, the PY form was selected due to its superior sensitivity to NO additions.

For physiological applications, preliminary studies relevant to physiological conditions were obtained in PBS (pH 7.4). Figure 1b shows NO calibration data for a CFE/PB/PoPD sensor; this configuration gave a sensitivity of ~5.3 A M$^{-1}$ cm$^{-2}$, an acceptable value for physiological applications. Response against interference was checked using nitrite ions (Figure 1c) and ascorbic acid, two of the most important interference species for NO sensors. These data revealed
that these configurations may be used in physiological applications without significant interference contributions.

In future work we plan to optimize these configurations and study in detail the most important analytical parameters for physiological applications. In addition, some *in-vivo* experiments in rat brain will be carried out to investigate its utility in the neuroscience field.

![Figure 1](image)

**Figure 1** a) Voltammogram evolution of PB modified electrode (screen printed) at different concentration of NO in acid medium. b) Calibration curve in phosphate buffer (pH 7.4) for a NO microsensor based on CFE/PB/PoPD configuration. c) Constant potential amperometry (CPA, 0.7 V) in PBS for a PB modified electrode (screen printed disks): (*) additions of 10 µM NO, (#) additions of 50 µM NO$_2^-$. After the addition of aliquots, the solutions were stirred for 10 s and then left to reach the quiescent steady-state current.

**Acknowledgment**

The funds for the development of this device have been provided by subprograma INNCORPORA and FEDER (INC-TU-2011-1621), Ministerio de Industria, Turismo y Comercio (TSI-020100-2011-169 and TSI-020100-2010-346); Ministerio de Ciencia e Investigación (TIN2011-28146).

**References**