BACKGROUND

From research performed over the past decade, nitrite and its precursor nitrate are currently viewed as storage pools for NO-like bioactivity, thereby representing an important alternative source of NO to the classical L-arginine-NO synthase pathway. The therapeutic potential of nitrate and nitrite in conditions such as myocardial infarction, stroke and pulmonary hypertension has emerged afterwards. Such discoveries turned the investigation of the mammalian nitrogen cycle a hot topic of research.

The actual levels of nitrogen oxides (NOx) in biological fluids have been difficult to measure because the currently used analytical assays do not provide enough selectivity and sensitivity to detect low nM concentrations in complex matrices. Moreover, these methodologies do not allow fast sampling processing, thus failing to preserve the original levels of NOx metabolites in samples (both NO and nitrite react rapidly with blood proteins under oxygenated conditions).

As so, this Master project is designed to deliver novel and effective alternative tools for nitrate/nitrite monitoring in physiological samples (blood and urine). Such innovative point-of-care test (POCT) could contribute, for example, to establish consistent associations between low nitrite levels and cardiovascular diseases (CVD), thus providing early alerts for these pathologies. Major progresses in the field were done in our group through the development of enzyme based electrochemical biosensors for nitrite quantification. Typically, the biosensors displayed high sensitivity, selectivity and very low detection limits.

OBJECTIVES

This research project aims at developing a disposable, low-cost and easy-to-use, integrated multi-electrodes system - NOxPROBE - for simultaneous quantification of nitrate/nitrite in physiological samples (blood and urine), at physiological pH and temperature, without sample preparation.

Well-established macroelectrode technology will be transferred to paper-based sensors, which can easily be produced in versatile configurations using inkjet and screen-printing technologies. The paper itself represents an economical and disposable substrate with an eco-friendly life-cycle.

For quantification of nitrite and nitrate, the biosensors will integrate selective redox enzymes within carbon paste/ink electrodes, on porous paper substrates. The transducing surface may be further modified with enhancers of enzyme activity (e.g. carbon nanotubes or polymeric layers to improve electrode protection and stability.)
PROJECT DESCRIPTION

Overall, the NOₓPROBE will encompass several working electrodes, sharing a common counter and reference electrodes. The analytical performance will be tailored according to the expected concentration range of each species, in each sample (e.g. nM nitrite in blood; mM nitrate in saliva).

The construction of paper multi-electrodes will be carried out at FCT, following recently implemented protocols, under co-supervision of Dr. Fortunato.

The sensors response to each analyte will be firstly examined by cyclic voltammetry; further analytical characterization (linear range, detection limit, selectivity, sensitivity and shelf-life) will be attained by amperometry, poising the electrodes at convenient potentials. The measurements will be done with a portable (multi)potentiostat which will be programmed for multi-sensing detection.

The NOₓPROBE will be tested in spiked samples and validated with the Griess method.

Finally, the NOₓPROBE will be used in epidemiological (clinical settings) studies relating basal nitrate/nitrite levels with CVD risk factors (e.g. hypertension, hypercholesterolemia).