Foreword

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Nitric oxide, NO, is one of the ten smallest molecules found in nature and interest in its measurement originated from studies of its role as an environmental contaminant in earth's air and water. However, the discoveries made in the 1980s that NO could be synthesized by mammalian cells and could act as physiological messenger and cytotoxic agent had elevated the importance of its detection. In this connexion, let's recall here that *R. F. Furchgott*, *L. J. Ignarro* and *F. Murad* received the 1998 Nobel Prize in Physiology or Medicine for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system. Thus, it appeared, and this can be easily deduced from the literature, that many of the numerous properties of NO – that enable it to carry out its diverses functions in the nervous, immune and cardiovascular systems - also present considerable problems when attempting its detection and quantification in biological systems. Since NO – which is a free radical – reacts very easily with oxygen, peroxides, O_2-radicals and metals (and metalloproteins), this explains its instability in biological systems. Detection of nitric oxide remains a challenge, pointing out the difficult dual requirements for specificity and sensitivity.

It is also known that superoxide anion, O_2^-, is one of the most important physiopathological modulator of local NO concentration in blood vessels. Its increased formation has been implicated in several cardiovascular and neurodegenerative diseases. Figure 1 shows a general view of NO and O_2^- vascular pathways, but the exact mechanism(s) by which superoxide mediates its cytotoxic effects are not clear yet. It remains a lot to explore and learn about the interactions between these two fascinating molecules in physiological and pathophysiological processes. However, evidence indicates that formation of superoxide-derived oxidants may also be involved. Indeed, superoxide generates other biological oxidants such as hydrogen peroxide by dismutation and peroxynitrite from the reaction with nitric oxide.

Among the thousands of publications investigating the biosynthesis of NO and O_2^- and their physiological effects, the percentage that includes estimations of their levels is extremely low (4 % for the past five years, in the case of NO). The numerous varieties of techniques for assessing nitric oxide and superoxide production, roles, properties and effects in biological systems explain the numerous publications and the abundance of international meetings and conventions that exist, regarding these subjects. Among the existing writings, that edited by M. Feelisch and J. Stamler entitled *Methods in Nitric Oxide Research* [1] is noticeable due to its detailed descriptions of specific methods for the measurement of NO, as well as techniques for immunolocalization and a variety of chemical, biochemical and spectroscopic approaches to its detection and to the determination of its redox related species. Among the international periodical scientific publications, it is important to mention the following journals: *Nitric Oxide*, *Free Radical in Biology and Medicine*, *Free Radical Research*, etc. and their periodical forums and follow-ups that are very useful in compiling a quick bibliography. Series like *Methods in Molecular Biology* (published by Humana Press) and *Methods in Enzymology* (published by Academic Press) offer also special issues devoted to protocols for the measurements of nitric oxide and oxygen radicals. The *Nitric Oxide Society* and *The Society for Free Radical Research* websites [2,3] provide updated calendars for the events related to this field and full information on the activities of several worldwide groups involved in these researches. The activity of the French scientists involved in this field is also well structured, especially *via* the *Club NO* [4].

Most methods currently available for detecting nitric oxide and superoxide are indirect and some of them prone to severe interferences and artifacts. The most commonly developed techniques are chemiluminescence, UV-visible spectroscopy, fluorescence, electron paramagnetic resonance spectroscopy (EPR) and electrochemistry. The goal of the *Characterization of Nitric Oxide and Superoxide in Biological Systems* "Dossier" published this month in ANALUSIS (European Journal of Analytical Chemistry) is to illustrate and specify the trends of the current developments in this field. The aspects discussed here are related to the various principles on which are based the techniques that have been developed for the detection of NO and O_2^-. While each technique has certain individual advantages, all of the techniques have disadvantages that limit their application. These disadvantages range from lack of sensitivity or specificity to interference from factors commonly present in biological systems. The choice of a technique is therefore dependent upon the desired applications.

In the case of nitric oxide, and exception made for the electrochemical techniques, most of the approaches use indirect methods for estimating endogenous NO, relying on measurements of secondary species such nitrite and nitrate or NO-adducts. They also suffer from allowing only *ex-situ* measurements. The Griess reaction, in which nitrite and nitrate ions, the main metabolites of NO, are chemically transformed into a colored diazo compound, constitutes the

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basis of the most widely used assays. Another commonly used method, the oxyhemoglobin reaction assay, uses the shift in the optical absorption spectra when oxyhemoglobin reacts with NO to form methemoglobin. EPR spectroscopy is also widely used for NO detection through the development of several methods of NO-trapping. Finally, gas phase chemiluminescence assay, based on the chemiluminescent properties of excited NO_2^* formed from NO that reacts with ozone, offers real utility and ease for some specific applications and may considered as the most reliable, rapid and reproducible assays available. This technique is now commercially available through NOA model 280 equipment commercialized by Sievers Research Company (Boulder, Colorado, USA [5]). But the only strategies that allow a direct and *in-situ* detection of NO are those based on the use of ultramicroelectrodes. Several NO-electrochemical microsensors have been developed to quantify nitric oxide production through its oxidation, by using amperometric or voltammetric techniques. Two NO-electrochemical sensors are now widely used, a nickel porphyrin and nafion® coated carbon microfiber (diameter of 7 µm) and a membrane-coated platinum (diameter 200 µm) or carbon (diameter 30 µm) electrode. This latter one is better known as the ISO-NO sensor commercialized by WPI Company (Sarasota, Florida, USA [6]).

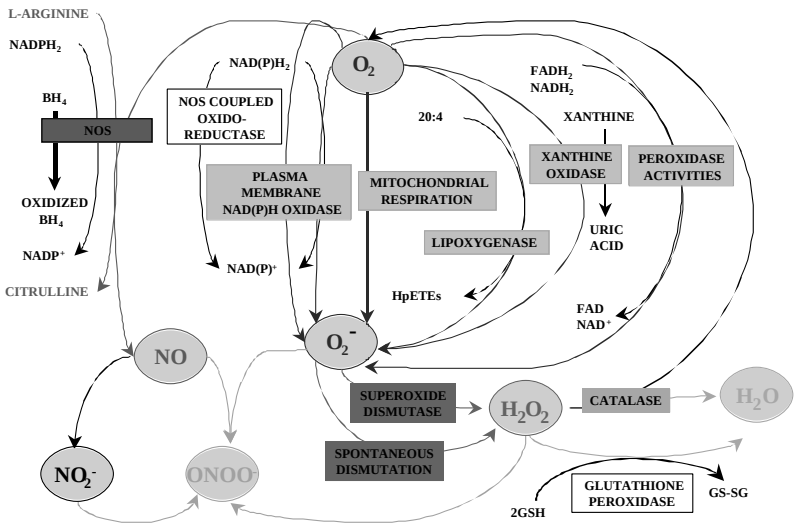

Figure 1. General description of vascular pathways of O_2^- (with permission from Iliou *et al*, see Iliou *et al*'s contribution for further details).

In the case of superoxide, the most reliable assays for its detection are based on the reduction of ferricytochrome *c* to generate ferrocytochrome *c* that is analyzed by UV-visible spectrophotometry. Chemiluminescence has also become very popular due to high sensitivity and reactivity of several chemiluminescent probes with superoxide. Lucigenin, an acridine-based compound, belongs to this class of superoxide detector. The reaction between lucigenin and superoxide results in the formation of an electronically excited dioxetane species that decomposes emitting light. Also, EPR technique is widely developed through the use of the spin-trapping approach that is based on the addition reaction of superoxide to an EPR-silent spin trap, usually a nitrone compound, to form an EPR-active adduct. None of these strategies allow direct and real time *in-situ* detection of superoxide in biological systems. The development of amperometric micro(bio)sensors, based on the oxidation of superoxide (or its main metabolite, hydrogen peroxide) is now reaching high levels of accuracy and allows the direct monitoring of its release and reactivity. None of these electrochemical techniques are commercially available. Finally, it should be noted here that this "Dossier" would not consider the various approaches devoted to the detection of related oxygen-derived oxidants such as hydrogen peroxide, hydroxyl radical, etc. which constitute a huge pool of investigations and will be briefly mentioned.

As the co-ordinator of this "Dossier", I have chosen to ask for contributions from several groups dealing with the different techniques of detection cited above. Therefore, this document consolidates articles concerning electrochemical, chemiluminescence, EPR, fluorescence and UV-visible spectrophotometry techniques, in which the different concepts and their comparison and improvements were analyzed and discussed. I would like to take here the opportunity to thank again all the authors for the high standard scientific level of their contributions and for their helpful efforts to constitute this "Dossier".

References

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