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Novel biocompatible hydrogel-based amperometric sensor for nitric oxide gas detection: towards a non-invasive device

Corinne Wartelle and Fethi Bedioui*

Laboratoire de Pharmacologie Chimique et Génétique, FRE CNRS n° 2463/U INSERM n° 640, Ecole Nationale Supérieure de Chimie de Paris, 11 rue Pierre et Marie Curie, 75231 Paris, cedex 05, France. E-mail: fethi-bedioui@enscp.jussieu.fr; Fax: 33 144 26 67 50

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The first example of a biocompatible hydrogel-based amperometric device has been elaborated for the detection of nitric oxide in gas phase.

The measurement of nitric oxide (NO) is important for the direct examination of its regulatory roles in biological systems since it participates in the control of major cell functions including reactivity, proliferation and apoptosis.[1] For example, an inappropriate formation or metabolism of this molecule may lead to the development of various cardiovascular dysfunctions.[2] Also, NO is made in the human and animal airway, in particular the nasopharynx. It is a useful marker of inflammation and asthma.[3–6]

Measuring NO from liquid samples is now well documented[7] and electrochemical methods are recognized as being the only analytical way to determine its local concentration in solution (without disturbing NO metabolism and the associated regulatory pathways).[8] However, no simple direct sensing device is available for the local detection of biologically produced NO in the gaseous phase. For instance, chemiluminescence[6] is the method of choice, but the widely developed assay does not allow simple and direct determination.

Our interest in designing electrochemical sensing devices for biological NO production in solution[8] encourages us to explore the possibility of developing a gas phase sensor. Herein we report for the first time on such an approach by assessing the amperometric detection of NO from gaseous phase. To do so, we propose the use of biocompatible hydrogel membrane,[9] an ionic conductor formed from kappa-carrageenan, deposited on a three-gold disk array electrode. This allows us to constitute the preliminary step in building up an integrated novel "solid state"-like amperometric NO gas sensor device.

The main body of the integrated device is made of a home-made three-planar disk (each 1 mm in diameter) array electrode produced as previously reported.[10] In brief, it is prepared by sputter deposition of gold onto a polycarbonate base with an intermediate titanium layer as adhesion layer. The ionically conducting hydrogel membrane is then deposited on the planar surface of the substrate (see below).

In a very first step we have assessed that the electrochemical characterization of a simple redox probe, namely $Ru(NH_3)_6^{3+}$, can be easily performed in the hydrogel layer upon contact of the electroactive complex with the membrane. The whole device was then completed by using a polycarbonate capsule mounted on the array of electrodes in order to create a localized restricted space to be partially filled by the hydrogel layer† where electrochemical processes take place at the gold disk electrode array surface underneath.

Fig. 1 shows the evolution of repeatedly recorded cyclic voltammograms of the hydrogel-containing device upon contact with 400 μL of $Ru(NH_3)_6^{3+}$ aqueous solution dropped over it, in the restricted space above the membrane. A well-defined couple of voltammetric peaks at *ca.* −0.4 V *versus* the pseudo gold reference electrode, related to the Ru^{III}/Ru^{II} redox process appears after 35 minutes of contact. Their intensities increase with time to reach a maximum 60 minutes later. Neither visible swelling of the hydrogel nor a phase mixing between the two phases were observed.

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This result be explained by a simple diffusion process of the cationic complex from its original aqueous solution into the polyanionic hydrogel.[11] This also allows to ensure that the three-electrode configuration, involving the gold pseudo reference, can be used with confidence. Indeed, the use of an external Ag/AgCl reference electrode allows the accurate location of the redox potential for the observed Ru^{III}/Ru^{II} process in the hydrogel. The obtained value, −0.2 V (data not shown), is not far from that obtained with the gold pseudo-reference electrode.

In a second step, we have tested the oxidation of NO by flowing a pure NO gas stream over the hydrogel. To do so, the chamber located above the hydrogel layer was tightly closed with a rubber cup through which airtight needles allow gas delivery. NO gas stream (98.5%; Aldrich) is first passed through a gas ramp previously purged with argon for 30 minutes. Then, it bubbles into a saturated KOH aqueous solution to eliminate all undesired NOx impurities. Finally, NO reaches *via* an oxygen-free pathway the electrochemical chamber and passes over and into the hydrogel solid membrane. Fig. 2 shows a drawing of the developed device.

Cyclic voltammograms recorded during NO flow showed the appearance of an anodic hump-like peak at *ca.* 0.75 V, with a neat increase in its intensity with time (data not shown). Upon establishing an argon atmosphere above the hydrogel, there is a complete disapperarance of the previously observed oxidation voltammetric peak. This allows to suggest that the observed peak at 0.75 V is related to the electrochemical oxidation of NO. This potential value was then applied for further chronoamperometric measurments.

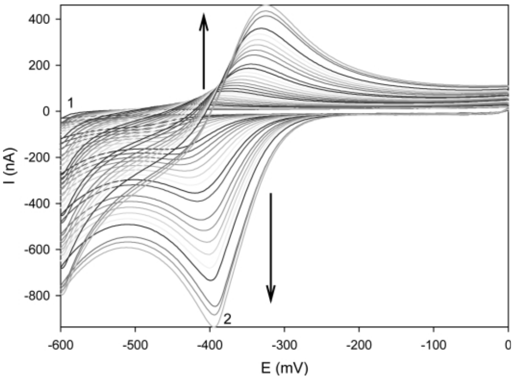

Fig. 1 Evolution of repeatedly recorded cyclic voltammograms of the hydrogel (*vs.* gold pseudo-reference electrode) in contact with 5 mM $Ru(NH_3)_6^{3+}$ aqueous solution. Curve 1: contact time = 1 minute; curve 2: contact time = 60 minutes. (Scan rate = 0.1 V s^{-1})

Fig. 3 shows the chronoamperometric measurement performed to confirm the usefulness of the elaborated device as an NO gas sensor. When the potential is stepped to 0.75 V and the gas chamber is fed with argon, the measured amperometric signal has a zero level (Phase 1). Upon streaming an NO gas flow (normal partial pressure of NO), an immediate sharp increase in the amperometric signal is observed, as is indicated in phase 2 of Fig. 3. Note that a decrease in NO flow rate (indicated by * in the graph) provokes a distinct slowing in the increase of the amplitude of the amperometric response. This also shows that the response time of the sensor is relatively short. By supplying argon gas instead of NO, an immediate sharp decrease of the amperometric signal is observed (phase 3 in Fig. 3). The current reaches zero level within 2 minutes. Such changes in amperometric current amplitudes under NO or argon atmosphere were reproducible (phases 4–7 in Fig. 3).

These results clearly indicate that the measured amperometric response is linked to the passage of NO gas through the hydrogel-based device. Although the sensor is not reaching steady state when the gas is switched back to NO (due to the short period of measurement under NO atmosphere of less than 5 minutes), it responds rapidly. Also, the modification of the amplitude of the recorded amperometric response by qualitatively changing the NO gas flow rates (phase 6 was performed with a much higher gas flow rate than used during phases 2 and 4) allows us to expect a usual gasp by gasp analysis.

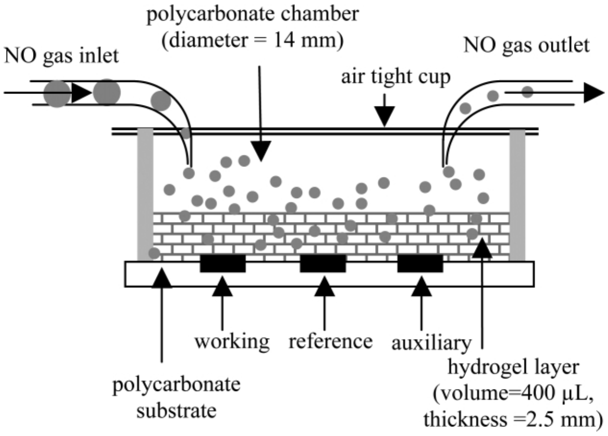

Fig. 2 Schematic drawing of the hydrogel "solid-state" electrochemical device for NO amperometric measurement

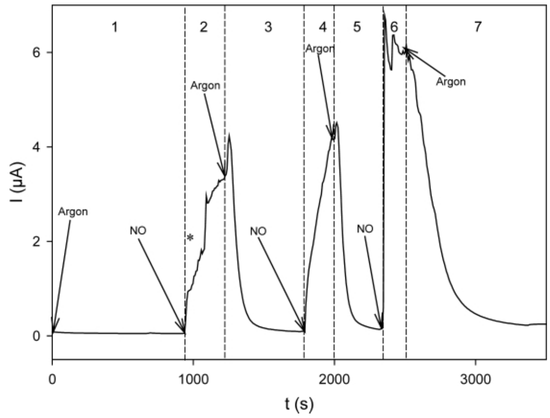

Fig. 3 Evolution of recorded chronoamperogram, at 0.75 V *vs.* gold pseudo-reference electrode, of argon and NO gas flowing alternately over the hydrogel in the studied device. Phase 1: argon flow; phase 2: NO gas flow (* indicates a reduction of the gas flow); phase 3: argon flow; phase 4: NO gas flow; phase 5: argon flow; phase 6: NO gas flow (with a higher gas flow rate) and phase 7: argon flow.

These descriptive results show the possibility of elaborating the amperometric detection of NO gas by simply using the hydrogel membrane on a three-gold disk electrode array. This unambiguously constitutes the first amperometric integrated hydrogel-based sensing device. Although these preliminary data do not thoroughly explore the analytical performance of the proposed tool (detection limit, sensitivity and time response), its applicability as a biocompatible-based sensing device is attractive. Also, the use of the hydrogel membrane flattened on a miniaturized disk electrode array may be suitable for the development of a flexible non-invasive sensor for small biologically important reactive species, such as NO in animal airways, or significantly hazardous or biologically important electroactive gas molecules.

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Notes and references

† *Representative procedure* for the preparation of the hydrogel: 115 mg of kappa-carrageenan (Sigma) was added to 10 mL of pure Milli-Q water, stirred and heated gently to 65 °C. When the liquid mixture is warm, 400 µL are pipetted above the electrode device and let to cool slowly in air for 10 minutes. The obtained clear hydrogel layer, composed of almost 98% water is then hard enough. It is stored at 4 °C overnight before use. It kept its original appearance and properties for at least 6 hours at room temperature, and four days at 4 °C.

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