

Design of electrochemical microsensors to monitor nitric oxide production in biological systems: a global compilation

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We report in this paper a global and exhaustive overview of the various attempts reported in the literature to develop electrochemical microsensors for the direct determination of nitric oxide (NO) in biological systems. In this compilation, we address the physico-chemical characteristics and the performances (in term of sensitivity) of the numerous chemically modified microelectrodes that were specially designed for the determination of NO, without judging what the biological applications might be.

Since the identification of nitric oxide, NO, as being the endothelial-derived relaxing factor, several research groups are working to develop an understanding of the mechanism by which this free-radical is synthesised, diffused and reacted in various biological systems [1-7]. It appeared that measuring NO in biological systems and/or models is very difficult because of its low concentration and fleeting existence. The desire to measure *in situ* very small amounts of NO release (in the submicromolar concentration range) has led to an active area of research involving the design of electrochemical microsensors [8-36].

Table I. Description of the NO-electrochemical microsensors.

Electrode	Modifier(s) (inner/outer layer)	NO-oxidation potential (/reference electrode)	Sensitivity (detection limit)	[Linear dynamic range; M] (experimental conditions)	Ref.
Carbon (fiber; $\phi=0.5 \mu\text{m}$; length= 2-6 μm)	NiTHMP ¹ / Nafion ²	0.64V /SCE	2.05 nA/ μM^c (10nM ^c ; 20 nM ^a)	[>30010 ⁻⁶] ^c (aerated PBS, pH=7.4)	[9]
Carbon (fiber; $\phi=8 \mu\text{m}$; length= 500 μm)	Cellulose ⁴	0.95V /Ag;AgCl	250-500 pA/ μM^c (~ μM)	[>10 μM] (aerated PBS, pH=7)	[10]
Carbon (fiber; $\phi=8\mu\text{m}$; length=2mm)	NiTHMP ¹ /Nafion ²	0.75V /SCE	6.28 nA/ μM^a (1.5nM) ^a	[1.510 ⁻⁹ -3.410 ⁻⁶] ^a (aerated PBS, pH=7.4)	[11]
Carbon (fiber; $\phi=30-35 \mu\text{m}$; length=100-150 μm)	Nafion ³ /o-PD ¹	0.775V /SCE	31 pC/ μM^d (35 nM) ^d	[10 ⁻⁷ -610 ⁻⁶] ^d (deaerated PBS, pH=7.4)	[12]
Carbon (fiber; $\phi=30-35 \mu\text{m}$; length=500 μm)	Nafion ² /(m-PD+resor.) ¹	0.7V /Ag;AgCl	0.5 nA/ μM^a (60-80 nM)	[0.2 10 ⁻⁶ ;110 ⁻⁶] (aerated PBS, pH=7.4)	[13]
Carbon (fiber; $\phi=30-40\mu\text{m}$; length=200-250 μm)	NiTHMP ¹ /Nafion ³ /AAO ⁴ / polylysine ⁴	0.65V /Ag;AgCl	1.34 pC/ μM^c (0.5 nM) ^c	[10 ⁻⁹ -10 ⁻⁴] (aerated PBS, pH=7.4)	[14]
Carbon (fiber; $\phi=8 \mu\text{m}$; length=0.8 mm)	NiTHMP ¹ /Nafion ³ Nafion ³ /o-PD ¹ Nafion ³ /(m-PD+resor.) ¹ NiTHMP ¹ /Nafion ³ /o-PD ¹	0.73-0.74V /SCE	14.5 nA/ μM^a 9.6 nA/ μM^a 4.9 nA/ μM^a 11.3 nA/ μM^a (≥ 27 nM) ^a	[410 ⁻⁸ -2 10 ⁻⁷] ^a (aerated PBS, pH=7.4)	[15]
Carbon (fiber; $\phi=8 \mu\text{m}$; length=0.8mm)	NiTSPc ¹ /Nafion ³	0.70V /SCE	11.3 nA/ μM^a (20 nM) ^a	[210 ⁻⁸ -210 ⁻⁷] ^a (aerated PBS, pH=7.4)	[16,17]
Carbon (disk $\phi=10 \mu\text{m}$)	CuPtCl ₆ ¹ /Nafion ⁴	0.70V /SCE	19.7 $\mu\text{A}/(\mu\text{Mcm}^2)^c$ (5 nM) ^a	[110 ⁻⁷ -210 ⁻⁴] ^c [110 ⁻⁸ -210 ⁻⁴] ^a (1M KCl; pH=7.4 0.025M; aerated)	[18,19]
Carbon (fiber; $\phi=8 \mu\text{m}$; length $\approx 1\text{mm}$)	Nafion ³ /o-PD ¹	0.76V /SCE	9.6 nA/ μM^a	[10 ⁻⁷ -510 ⁻⁷] ^a (deaerated PBS, pH=7.4)	[20]
Glassy carbon (disk; $\phi=3 \text{mm}$)	NiTMPyP ¹ /Nafion ² NiTSPp ¹ /Nafion ²	0.7V /SCE	n.r. (0.25 μM) ^a	[710 ⁻⁶ -2.510 ⁻⁵] ^a (pH=7.4 +EDTA, glucose ; NO ₂ ⁻ , deaerated)	[21]
Glassy Carbon (disk; $\phi=30 \mu\text{m}$)	(PMePy,FeHPA) ¹ / Nafion ¹	0.72V /Ag;AgCl	2.65 nA/ μM^a (100 nM) ^a	[10 ⁻⁷ -10 ⁻³] ^a (deaerated PBS, pH=7.4)	[22]
Glassy carbon (disc; $\phi=n.r.$)	NiTSPc ⁴ /Nafion ⁴	0.80V/SCE	n.r. (~10 nM) ^a	[110 ⁻⁸ -1.210 ⁻⁷] ^a (aerated PBS, pH=7.0)	[23]
Glassy carbon (disc; $\phi=n.r.$)	NiTHMP ¹ / (Nafion ² or Eugenol ¹)	0.75V-0.80V /Ag;AgCl	10.5 nA/ μM^d (85 nM) ^d	[0.5-2010 ⁻⁶] ^d (aerated PBS, pH=7.0)	[24]
Glassy carbon (disc; $\phi=2\text{mm}$)	CA ⁴	0.90V /Ag;AgCl	n.r. (20 nM) ^d	[>100 10 ⁻⁹] (0.1 mol/L PBS, pH=7.4 aerated and deaerated)	[25]

Table I. Continued.

Electrode	Modifier(s) (inner/outer layer)	NO-oxidation potential (/reference electrode)	Sensitivity (detection limit)	[Linear dynamic range; M] (experimental conditions)	Ref.
Glassy Carbon (disk; $\phi=3$ mm)	CuPtBr ₆ ¹	0.79V /SCE	n.r. (20 nM) ^a	[510 ⁻⁸ -110 ⁻⁴] ^a (1M KCl ; pH=7.4, 0.025M ; deaerated)	[19,26]
Pt (fiber; $\phi=150$ -250 μ m)	Neoprene/NaCl/KCl	0.9V /Ag; AgCl	3-100 pA/ μ M ^d (8nM) ^d	[1-310 ⁻⁶] ^d (n.r.)	[27]
Pt (fiber; $\phi=200\mu$ m: length=n.r.)	KCl/nitroC/Silicone	0.4V /graphite	0.23pA/nM ^d (6.5nM) ^d	[0.210 ⁻⁹ -110 ⁻⁶] ^d (aerated SNAP; pH=[6-8.5]; NaCl 0.1 M or aerated SNAP+KH)	[28]
Pt (disc; $\phi=75\mu$ m)	Nafion/CA	0.9V /SCE	n.r. (100 μ M) ^b	[10010 ⁻⁶ -50010 ⁻⁶] ^b (aerated PBS, pH=7.4)	[29]
Pt (disc; $\phi=2$ mm)	Poly 4-4'DHB ¹ Nafion ³	0.75 /Ag;AgCl 0.90 /Ag;AgCl	n.r. (40 nM) ^d (80 nM) ^d	[>10010 ⁻⁹] (0.1mol/L PBS, pH=7.4 aerated and deaerated)	[25]
Pt (disc; $\phi=1.6$ mm)	CytC-PITO ⁴ /Nafion ⁴	0.75V /Ag;AgCl	0.25 μ A/(μ M.cm ⁻²) ^d	[0.05-100 10 ⁻⁶] (pH=7; PBS 0.1M; aerated)	[30]
Pt (disc; $\phi=15$ μ m)	polyCoTAPc ¹ /Nafion ⁴	0.70V /SCE	n.r. (10 ⁻⁷ M) ^c	[210 ⁻⁷ -4.110 ⁻⁶] ^c (pH=5; deaerated 0.1 mol/l d'EDTA)	[31]
Pt (fiber; $\phi=15$ μ m)	Nafion ⁴ /PVP ¹ /(Pd/ IrO ₂) ¹	0.667V /SCE	n.r. (1.510 ⁻⁸ -510 ⁻⁸ M) ^a	[5.810 ⁻⁸ -6.410 ⁻⁶] ^a (deaerated PBS, pH=7.4, 0.1M)	[32]
Pt (disc; $\phi=200$ μ m)	Ni(ABED) ⁴ /Nafion ²	0.7V /SCE	6.5 nA/ μ M ^a (5 nM) ^a	[110 ⁻⁸ -110 ⁻⁶] ^a (aerated PBS, pH=7.4)	[33]
Pt (disc; $\phi=15\mu$ m)	Ni ¹ (chitin) ₂ ⁴ /Nafion ⁴	0.74V /SCE	n.r. (50 nM) ^a	[8.510 ⁻⁸ -1.510 ⁻⁵] ^a (aerated PBS, pH=7.4; +NaCl 0.137 M+KCl 0.008M)	[34]
Pt (fiber; $\phi=15$ μ m)	M(salen) ¹ /Nafion ⁴	[0.68-0.71] /Ag;AgCl	n.r. (5-20 nM) ^a	[19.6 10 ⁻⁹ -4010 ⁻⁶] ^a (aerated PBS, pH=7.4)	[35]
Gold (disk: $\phi=25\mu$ m/ fiber: $\phi=25\mu$ m; length=0.5-1 cm)	Nafion ²	0.76V /SCE	n.r. (n.r.)	[1010 ⁻⁹ -10010 ⁻⁶] ^c (deaerated; glucose PBS+NaCl+CaCl ₂ ; pH=7.4)	[36]

n.r.: not reported ;

1: electrochemical deposition; 2: dipping and drying by evaporation; 3: dipping followed by thermal treatment; 4: droplet deposition and drying by evaporation.

a- differential pulse amperometry; b- cyclic voltametry; c- differential pulse voltametry; d- amperometry.

(AAO=ascorbic acid oxidase; m-PD= *meso*-phenylenediamine; o-PD= *ortho*-phenylenediamine; resor.=resorcinol; NiTHMP=tetrakis(3-methoxy-4-hydroxyphenyl)nickel(II) porphyrin; NiTMPyP=tetrakis(N-methylpyridinium)nickel(II) porphyrin; NiTSP=tetrakis(4-sulfonatophenyl)nickel(II) porphyrin; NiTSPc=tetrasulfonato(nickel(II)) phtalocyanine; PMePy= poly(N-methylpyrrole); FeHPA=iron substituted Keggin-type heteropolyanion or [H₂OFe(III)PW₁₁O₃₉]; CA=cellulose acetate; PVP= poly(vinyl pyridine); poly4-4'DHB=poly[4,4'-dihydroxybenzophenone]; CytC-PITO= cytochrome C associated to 2-(-4-carboxyphenyl)-4,4,5,5-tetra methyl imidazoline-1-oxyl-3-oxide; M(salen)=metal ethylenebis(salicylideneiminato); Ni(ABED)=*ortho*-aminobenzaldehyde-ethylene-diamine nickel; polyCoTAPc=cobalt(II)tetraaminophthalocyanine; nitroC=nitrocellulose; Ni(chitin)₂=nickel(II)chitin; PBS: phosphate butter solution; SCE: saturated calomel electrode).

In fact, one of the challenges in developing electrochemical microsensors is the necessity to have a simple, sensitive, selective and easy-to-handle tool that offers a rapid response time and a long term stability. The explosive growth in the fabrication and the high levels of sophistication of the design of ultramicroelectrodes, with extremely small dimensions, strongly stimulates this strategy and contributes to promoting their application in the field of the direct and local NO detection.

The reality is that surface electrode modification is needed to make the ultramicroelectrode material selective for NO. Therefore, the design of modified electrode surfaces using organised layers is very attractive and may provide the ideal strategy. In the general case, the chemical modification of electrode surfaces with polyelectrolytes and metal complex-based polymer films has expanded the scope of application of such new designed electrodes and provided a lot of options for their use in various experimental conditions. In addition to their electrocatalytic applications [37], such electrodes showed a great promise for electroanalysis [38,39]. Indeed, electroanalysis with polymer-coated electrodes can benefit from their catalytic and discriminative transport properties [40-45]. As far as this aspect is concerned, substantial improvements in selectivity, sensitivity, versatility and reproducibility can be achieved.

The major goal of the reported attempts in designing NO microsensors has been to improve the selectivity and the sensitivity of the electrochemical measurement of NO through sensor surface modification. For this purpose, additional polymeric membranes are deposited onto platinum, gold, glassy carbon or carbon fibre or disk electrode surfaces [9-36]. An elegant method of electrode modification is the electropolymerization of suitable monomers. Progress in this area is strongly connected to the design of new electroactive polymeric systems and to the success in forming thin, insoluble, stable, reproducible and adherent films on electrode surfaces. The major additional advantage of the electropolymerization design is the ability to coat very small or irregularly shaped electrode surfaces. In the case of non-conducting polymer films, the electropolymerization process also offers the ability to control the film thickness, via the amount of charge passed, by self regulation, since they would grow thick enough to become insulators.

Table I contains a global and exhaustive up-dated compilation of the various attempts reported in the literature to develop electrochemical microsensors for the direct determination of nitric oxide in biological systems. In this compilation, we address the physico-chemical characteristics and the performances, in term of sensitivity to NO, of the numerous chemically modified microelectrodes that were specially designed for the determination of NO, without judging what the biological applications might be. It appears from this description that the use of chemically modified microelectrodes approach provides an elegant way to build up multi-layered structures that result in NO-sensors with real high performance characteristics and offers new deals to further evaluate their validity. Exception made for the commonly

used and most developed nickel porphyrin and Nafion®-based sensor, no definitive conclusions can be drawn concerning the performances and the use of the various microsensors in real biological systems, due to the lack of published results. Anyhow, these examples constitute a huge pool of possible and perfectible molecular materials suitable for the electrochemical sensing of NO.

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