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ORIGINAL PAPER

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Calibration of NO sensors for in-vivo voltammetry: laboratory synthesis of NO and the use of UV–visible spectroscopy for determining stock concentrations

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Abstract The increasing scientific interest in nitric oxide (NO) necessitates the development of novel and simple methods of synthesising NO on a laboratory scale. In this study we have refined and developed a method of NO synthesis, using the neutral Griess reagent, which is inexpensive, simple to perform, and provides a reliable method of generating NO gas for in-vivo sensor calibration. The concentration of the generated NO stock solution was determined using UV–visible spectroscopy to be 0.28 ± 0.01 mmol L^{-1}. The level of NO_2^- contaminant, also determined using spectroscopy, was found to be 0.67 ± 0.21 mmol L^{-1}. However, this is not sufficient to cause any considerable increase in oxidation current when the NO stock solution is used for electrochemical sensor calibration over physiologically relevant concentrations; the NO sensitivity of bare Pt-disk electrodes operating at +900 mV (vs. SCE) was 1.08 nA μmol^{-1} L, while that for NO_2^- was 5.9×10^{-3} nA μmol^{-1} L. The stability of the NO stock solution was also monitored for up to 2 h after synthesis and 30 min was found to be the time limit within which calibrations should be performed.

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Introduction

Nitric oxide (NO) is a gaseous, paramagnetic free radical, and is one of the smallest molecules found in nature. Although awareness in NO first developed due to its role as an environmental pollutant in relation to acid rain [1], air pollution [2], and destruction of the ozone layer [3], interest in NO rapidly increased in the 1980s with the discovery that NO is the endothelium-derived relaxing factor [4–6]. Since then NO has been recognised to be of immense physiological and pathophysiological importance. It regulates vascular tone, acts as a neuronal signal in the gastrointestinal tract and central nervous system, and contributes to the pathology of several diseases including hypertension, Parkinson's disease, and Alzheimer's disease. Thus, from a biochemical and medical perspective, it is important to quantify levels of NO production in abnormal and normal tissues using direct measurements. Current techniques used for direct measurement include spectroscopic (e.g. chemiluminescence and UV–visible spectroscopy) and electrochemical methods (e.g. Clark electrode and porphyrinic sensors). Of these techniques, electrochemical methods are most advantageous because of their speed and sensitivity [7–9].

For a number of years we have been using in-vivo voltammetry, i.e. detection of substances using amperometric electrodes (sensors) and electrochemical techniques in vivo, to study neurochemical phenomena in the living brain [10, 11]. By implanting a sensor in a specific brain region, applying a suitable potential profile and recording the resulting Faradaic current, changes in the concentration of a variety of substances in the extracellular fluid can be monitored with sub-second time resolution over extended periods. This enables

investigation of the functions and roles of specific neurochemicals in neuronal signalling, drug action, and well-defined behaviour, with a high time resolution not presently available with other methods. We have recently become interested in developing a new sensor for monitoring brain extracellular levels of NO. As a first step in this process we have developed a novel permselective membrane coating to eliminate interference signals [12], because in designing an in-vivo sensor one must maximise not only speed and sensitivity but also interference rejection of other endogenous electroactive species. This is particularly important for NO, because it is present at nanomolar concentrations and has a half-life of 2–6 seconds in vivo [13].

To enable accurate calibration of our new NO sensor it was first necessary to establish and characterise a consistent and reproducible method of preparation of NO stock solutions. There are various methods of preparing NO on a laboratory scale. It was first synthesised and discovered in 1772 by John Priestly, by reacting nitric acid with copper metal [14]:

$$3Cu + 8HNO_3 \rightarrow 2NO + 3Cu(NO_3)_2 + 4H_2O$$

The NO produced by this reaction has been found to be relatively impure [15]. Four methods are currently commonly used to generate NO for sensor calibration. The first method involves the preparation of NO standard solutions from pure NO gas obtained by buying a commercial cylinder with a stainless-steel regulator. However, although this is the most commonly used method for NO sensor calibration found in the literature [16–22], it suffers from a number of drawbacks including expense and shelf-life.

A second method involves generating the NO in the actual calibration solution itself, by reduction of NO_2^- [23, 24]:

$$2KNO_2 + 2KI + 2H_2SO_4 \rightarrow 2NO + I_2 + 2H_2O + 2K_2SO_4$$

Although this is a straightforward method, the caustic nature of the acid makes it an unsuitable calibration procedure for most NO sensors [25].

A third method of NO production and calibration has been developed by World Precision Instruments (WPI) and involves the decomposition of *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP), an NO donor. SNAP in the presence of a catalyst, e.g. Cu(I), releases NO. This method of NO calibration is generally accepted as being suitable for most NO sensors [25–29]. However, SNAP does have drawbacks relating to its stability and purity, because it is extremely sensitive to light and temperature [21, 25, 30, 31].

It has been reported in the literature by Vilakazi and Nyokong [32] and by Fan et al. [33] that NO_2^- disproportionates into NO at pH less than 4. This is the basis of a fourth method of NO synthesis in which a concentrated H_2SO_4 solution is used to carry out the disproportionation using $NaNO_2$ as a source of NO_2^- [34, 35]. The NO gas thus generated is then bubbled into distilled H_2O generating an aqueous NO stock solution. In this paper we report the development of a modified form of this synthetic method which includes the design and use of a novel synthesis apparatus, purification steps involving N_2 purging, alkaline pyrogallol and KOH, and accurate determination of the concentration of contaminants. The resulting method is inexpensive, simple to perform, and provides an accurate means of generating NO gas for in-vivo sensor calibration.

In addition, while reviewing the literature it became apparent that many research groups working with NO standard solutions used no experimental method to verify the concentration of their stock solution, instead quoting saturation concentrations found in the literature — ca. 1.9 mmol L^{-1} at room temperature [19, 36–40]. Because of the reactivity of NO with molecular oxygen, and its high permeability characteristics [41], it should be considered important for research groups working with NO to verify their stock concentration. Thus, we also report the application of a photometric method to verify the concentration of the stock NO solutions generated.

Materials and methods

Reagents and solutions

Reagent-grade sodium nitrite, potassium hydroxide, *N*-1-naphthylethylenediamine (NEDD), and sulfanilamide (SULF) were obtained from Sigma–Aldrich Ireland (Dublin, Ireland). Orthophosphoric acid (85%) and sulfuric acid (97%) were obtained from BDH Chemicals, Poole, UK. Pyrogallol (1,3,5-trihydroxybenzene) was obtained from Hopkin and Williams, Essex, UK.

Phosphate-buffered saline (PBS) solution, pH 7.4, was prepared using deoxygenated doubly distilled deionised water (0.15 mol L^{-1} NaCl, 0.04 mol L^{-1} NaH_2PO_4 and 0.04 mol L^{-1} NaOH), and was deaerated with O_2-free N_2 for 20 min prior to commencing experiments.

UV–visible spectroscopy of NO

A 1:42 ratio of NEDD (0.4 mmol L^{-1}) to SULF (17 mmol L^{-1}) was used to determine NO stock concentrations. Neutral Griess reagent was made by dissolving NEDD and SULF in 100 μmol L^{-1} PBS (pH 7.4) by a process of stirring and gentle heating (approximately 10 min). A quartz crystal UV cuvette (Sigma–Aldrich Ireland) of 1 cm path length was used for UV analysis of NO stock solutions. Aerated Griess reagent (clear solution) was placed in the cuvette using a micropipette (Sealpette; Jencons Scientific, PA, USA), and an aliquot of NO stock solution was added to this using a gas-tight syringe (Hamilton Gastight 1,700 Series Syringe; Sigma–Aldrich Ireland). The cuvette was then inverted a couple of times. The orange azo dye

became apparent in the cuvette upon addition of NO and exhibited max intensity after 10 min (see Results and discussion, Analysis of NO stock solution below).

A Varian–Cary 50 UV–visible spectrometer was used in the determination of λ_{max} for the orange azo dye. The cuvette containing the dye was placed in the spectrometer and the wavelength range 400–600 nm was scanned to verify λ_{max}. A value of 496 nm was obtained which is in good agreement with the literature [42]. The concentration of the NO stock solution was determined by observing the absorbance obtained at 496 nm and using the Beer–Lambert law with an extinction coefficient of 12,500 mol^{-1} L cm^{-1} [42].

Electrochemical sensor preparation

Pt-disk electrodes were made from Teflon-insulated platinum/iridium (Pt/Ir 90%/10%) wire (125 μm bare diameter, 160 μm coated diameter (5T), Advent Research Materials, Suffolk, UK). The electrodes were approximately 5 cm in length and were prepared by carefully cutting 5 mm of the Teflon insulation from one end of the wire. A gold electrical contact (Semat Technical, Herts, UK) was soldered to this end of the wire to enable connection with the instrumentation. The other end of the wire acted as the active (disk) surface of the electrode. When not in use all electrodes were stored at room temperature.

NO calibration

NO calibrations were performed in a standard three-electrode glass electrochemical cell which was constructed in-house. A saturated calomel electrode (SCE) was used as the reference electrode and a large Pt wire served as the auxiliary electrode. To facilitate mixing, solutions were stirred using a magnetic stirrer and stirring bead for ca. 5 s after addition of each aliquot. The current was then measured when the solution was quiescent. The electrodes were held at a constant potential of +900 mV [19]. All calibrations were performed at room temperature.

Instrumentation and software

Constant-potential amperometry was performed in all electrochemical experiments using a low-noise potentiostat (Biostat II, Electrochemical and Medical systems, Newbury, UK). Data acquisition was carried out with a Gateway GP6-350 computer, a Powerlab/400 interface system (ADInstruments, Oxford, UK) and Chart for Windows (Version 4.0.1) software (ADInstruments).

All analysis was performed using Microsoft Excel. Data are represented as mean ± S.E.M. The significance of differences observed was estimated using the Student's *t*-test (two-tailed) for paired observations (InStat Version 3.0.5, GraphPad Software, CA, USA).

Results and discussion

Synthesis of NO

NO for in-vivo sensor calibration was synthesised in-house by modification of methods previously reported by Zhang et al. [31, 35], Vilakazi and Nyokong [32, 43] and Ge et al. [44]. Deoxygenated doubly-distilled deionised water (5 mL) was transferred to a Quickfit 10-mL round-bottomed flask (containing a magnetic stirring bead) which was then sealed with a rubber septum. This flask, which served as the NO collection vessel, was placed in a sonic bath at 25°C for approximately 10 min, and then connected to the NO-generation apparatus (Fig. 1), which had been purged with N_2 for 30 min before commencement of the synthesis. N_2 was also continuously purged during the synthesis by bubbling through a Drescher bottle

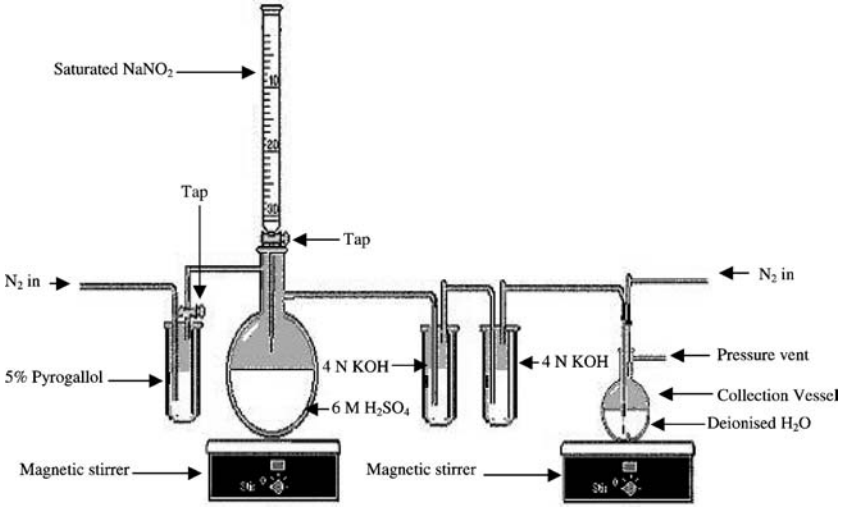

Fig. 1 Schematic diagram of apparatus used for generating the clear colourless NO gas. Because NO gas is toxic at concentrations higher than 100 ppm, and is highly reactive with molecular O_2, the synthesis is performed in a fume hood under a N_2 atmosphere. For details see Synthesis of NO in text

(200 mL) containing 5% alkaline pyrogallol (150 mL) to facilitate removal of trace amounts of O_2 that might be present. High-vacuum grease (Dow Corning, MI, USA) and Parafilm (American National Can, CT, USA) were applied to all Quickfit joints to minimise the possibility of O_2 entering into the apparatus. Such procedures created an inert atmosphere critical for NO synthesis [45].

NO gas was synthesised by reaction of saturated $NaNO_2$ with 6 mol L^{-1} H_2SO_4. The saturated $NaNO_2$ was dripped slowly from a Quickfit dropping funnel into a Quickfit round-bottomed flask containing the 6 mol L^{-1} H_2SO_4 (50 mL). A magnetic stirrer was used to promote the reaction (Fig. 1). It was clear from the colour of the resulting gas produced that NO was not the only oxide of N_2 generated; a rust colour indicated the presence of nitrogen dioxide (NO_2). The gas was then passed through two 200 mL Drescher bottles containing 4 mol L^{-1} KOH solution to completely remove any higher oxides such as NO_2 [35]. It was then passed into the NO collection vessel and the magnetic stirrer was used to continuously mix the deionised water during the collection, which lasted 15 min (see Analysis of NO stock solution below).

Analysis of NO stock solution

Two photometric techniques are routinely used to verify the concentration of stock NO solutions. The first is based on the acidic Griess reagent assay [46–49] and the second on the conversion of oxyhaemoglobin to methaemoglobin by NO [50–52]. However, both methods have inherent difficulties. One of the major obstacles in analysing NO via the Griess reagent is that during the preparation of NO stock solutions NO_2^- can be formed by exposure of the aqueous NO stock solution to O_2 during preparation. The principal difficulty confronting research groups performing photometric determination of NO by oxidation of oxyhaemoglobin is that commercial oxyhaemoglobin must be purified when purchased by reduction of the haem protein with dithionite or ascorbic acid, followed by desalting using sizing columns or overnight dialysis.

A more straightforward and simple methodological approach to the measurement of NO in neutral aqueous solution, which is not affected by nitrite, was devised by Nims et al. [42]. They used commercially available reagents along with UV–visible spectroscopy. After completing the NO synthesis the stock concentration in our sample bottle was determined using this method [42]. Briefly, when NEDD is dissolved with SULF in 100 μmol L^{-1} PBS (pH 7.4) under aerobic conditions, addition of stock NO solution results in visible absorbance at 496 nm. A primary arylnitrosamine is produced from the nitrosation of SULF by the intermediate generated in the NO/O_2 reaction [47]:

$$2NO + O_2 \rightarrow NO_X$$

The arylnitrosamine decomposes rapidly to a diazonium ion which reacts with NEDD to create an orange azo dye. UV–visible spectroscopy was used to determine λ_{max} (496 nm) for this dye, and the concentration of the NO stock solution was calculated using the Beer–Lambert law (for further details see Materials and methods, UV–visible spectroscopy of NO):

$$[NO]_{stock} = \frac{Abs}{\varepsilon NO} \times \text{dilution factor},$$

where $\varepsilon NO = 12{,}500$ mol^{-1} L cm^{-1} [42]. The mean concentration was calculated to be 0.28 ± 0.01 mmol L^{-1} ($n = 3$; 1:100 dilution).

We next decided to examine the relationship between volume of NO added to the neutral Griess reagent and absorbance at 496 nm. A stock NO solution was produced by the experimental procedure outlined above. Specific aliquots of NO were taken from this stock solution and injected into the UV cuvette containing the neutral SULF/NEDD solution. Figure 2 shows typical UV scans performed from 400 to 600 nm and the volume–absorbance profile for NO in SULF/NEDD solutions. In agreement with previous studies [42] these results demonstrate that the absorbance values obtained at 496 nm are directly proportional to the volume of NO stock solution added to the neutral Griess reagent ($R^2 = 0.98$).

This was followed by an investigation of the relationship between addition of NO to the SULF/NEDD solution and maximum NO-mediated orange colour development at 496 nm. The changing absorbance of the NO-mediated orange azo dye was monitored over 15 min. The results, shown in Fig. 3a, indicate that measurement of maximum absorbance using UV–visible spectroscopy is achieved after 10 min, with no signifi-

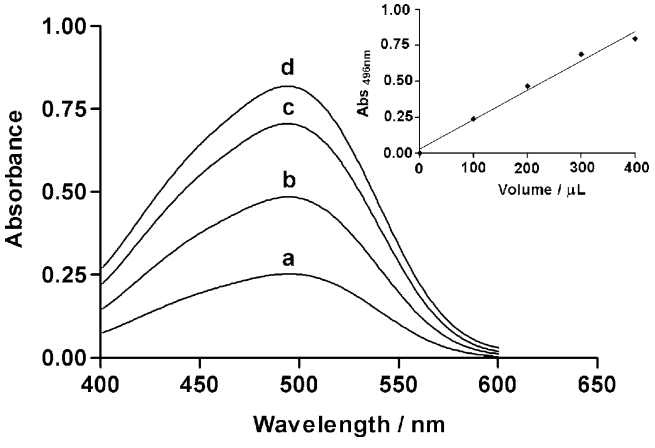

Fig. 2 Typical UV–visible scans from 400 to 600 nm profiling the relationship between absorbance at 496 nm and increasing NO concentration in SULF/NEDD solutions (17/0.4 mmol L^{-1} in 100 μmol L^{-1} PBS, pH 7.4); increases in absorbance represent injections of 100 (*a*), 200 (*b*), 300 (*c*) and 400 (*d*) μL NO into the SULF/NEDD solution. *Inset:* The volume–absorbance (496 nm) relationship for NO in SULF/NEDD solutions (17/0.4 mmol L^{-1} in 100 μmol L^{-1} PBS, pH 7.4), $R^2 = 0.98$, $n = 3$; 1:100 dilution

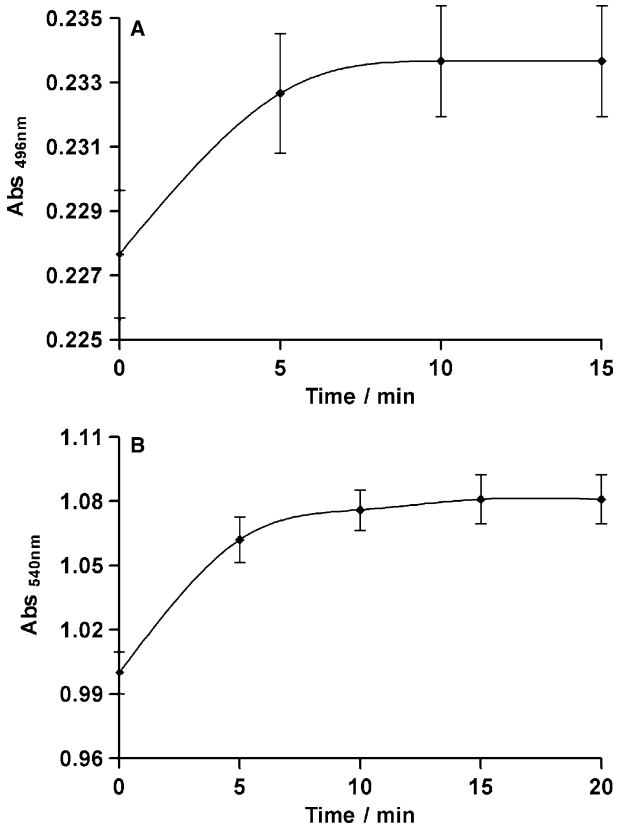

Fig. 3 **A** Time course of changes in absorbance at 496 nm after addition of NO to SULF/NEDD (17/0.4 mmol L^{-1} in 100 μmol L^{-1} PBS, pH 7.4) solutions (neutral Griess reagent), $n=3$; 1:20 dilution. **B** Time course of changes in absorbance at 540 nm after addition of NO_2^- to SULF/NEDD (17/0.4 mmol L^{-1} in 100 μmol L^{-1} PBS, pH <2.4) solutions (acidic Griess reagent), $n=3$; 1:100 dilution

cant change in absorbance thereafter. All 496 nm absorbances were thus measured at 10 min.

The relationship between NO collection time and NO concentration was also examined. Times of 15, 30, and 45 min were chosen for production of NO stock solutions. The absorbance at 496 nm was recorded (10 min) for three separate NO stock solutions (1:100 dilution) for each of 15, 30, and 45 min and used to calculate the concentration of the NO stock solution using the Beer–Lambert law (see above). No significant difference was observed between the concentration determined for 15 min (0.28 ± 0.01 mmol L^{-1}, $n=3$), and the concentrations for 30 (0.31 ± 0.03 mmol L^{-1}, $n=3$, $P=0.39$) and 45 (0.24 ± 0.03 mmol L^{-1}, $n=3$, $P=0.14$) min. It was thus decided to use a collection time of 15 min to produce our NO stock solutions.

Interference studies

Because the concentration of saturated NO solution at room temperature has been reported to be 1.9 mmol L^{-1} in the literature [19, 36–40] we decided to examine the possibility that our stock NO solution contained species other than NO which might be preventing a saturated concentration being obtained.

The high reactivity of NO with O_2:

$$4NO + O_2 + 2H_2O \rightarrow 4HNO_2$$

directed us toward testing for the possibility of NO_2^- contamination of our stock NO solution.

Ridnour et al. [47] had previously used a spectrophotometric method for the direct quantification of NO, NO_2^-, and NO_3^-. Acidification (pH 2.4) of a reaction involving NO, sulfanilic acid and NEDD generated a pink product absorbing at 540 nm which enabled complete determination of NO_2^- levels in the assay solution. An investigation, for verification purposes, of UV–visible absorbance at increasing NO_2^- concentration yielded a λ_{max} of 540 nm for the acidic Griess reagent (Fig. 4). To determine the relationship between absorbance at 540 nm and NO_2^- concentration it was first necessary to use NO_2^- standards to obtain a value for the molar absorbtivity (ε), to enable determination of contaminant concentrations in our NO stock solutions using the Beer–Lambert law. A direct proportionality ($R^2=0.99$) was observed with a slope of 139,000 mol^{-1} L cm^{-1} (ε) (inset Fig. 4).

Examination of the relationship between addition of NO_2^- to the acidic SULF/NEDD solution and maximum NO_2^- -mediated purple colour development at 540 nm indicated that maximum absorption occurs after 15 min (Fig. 3b). The concentration of potential NO_2^- contaminant in our NO stock solution was thus determined by measuring absorbance at 540 nm 15 min after the addition of NO stock solution aliquots to the acidic

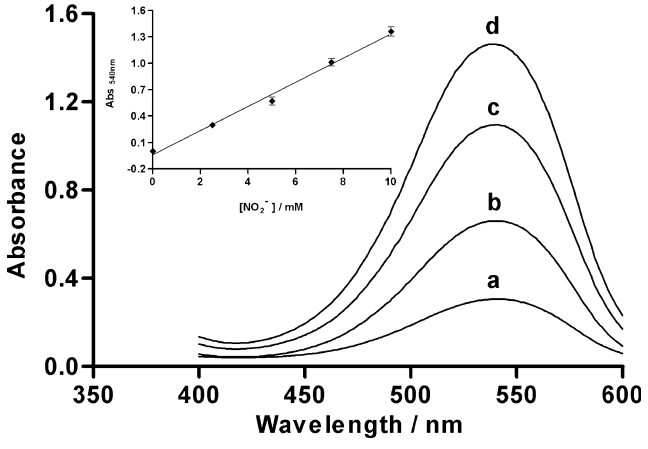

Fig. 4 Typical UV–visible scans from 400 to 600 nm profiling the relationship between absorbance at 540 nm and increasing NO_2^- concentration in SULF/NEDD solutions (17/0.4 mmol L^{-1} in 100 μmol L^{-1} PBS, pH $<$ 2.4); increases in absorbance represent injections of 2.5 (*a*), 5.0 (*b*), 7.5 (*c*) and 10.0 (*d*) μmol L^{-1} NO_2^- into the acidic SULF/NEDD solution. *Inset:* Relationship between absorbance at 540 nm and increasing NO_2^- concentration in SULF/NEDD (17/0.4 mmol L^{-1} in 100 μmol L^{-1} PBS) solutions with pH $<$ 2.4 (acidic Griess reagent), 139,000 mol^{-1} L cm^{-1} (slope), $R^2=0.99$, $n=3$; 1:100 dilution

Griess reagent. The average NO_2^- concentration determined for three stock solutions was 0.67 ± 0.21 mmol L^{-1} ($n = 3$; 1:100 dilution).

Electrochemical sensor studies

Although our NO stock solution clearly contains NO_2^- contaminant, it is not sufficient to cause any considerable increase in oxidation current when the NO stock solution is used for electrochemical sensor calibrations. The sensitivity to NO_2^- of bare Pt-disk electrodes operating at +900 mV (vs. SCE) was determined from calibrations (Fig. 5a) to be 5.90 ± 0.79 nA mmol^{-1} L ($n = 4$), while the sensitivity for NO, determined for physiologically relevant concentrations, was 1.08 ± 0.21 nA μmol^{-1} L ($n = 28$; Fig. 5b). Thus, for a 1 μmol L^{-1} calibration concentration of NO the corresponding concentration of NO_2^- would be ca. 1.69 μmol L^{-1}. This would result in an interference current of ca. 0.01 nA, corresponding to <1% of the NO signal.

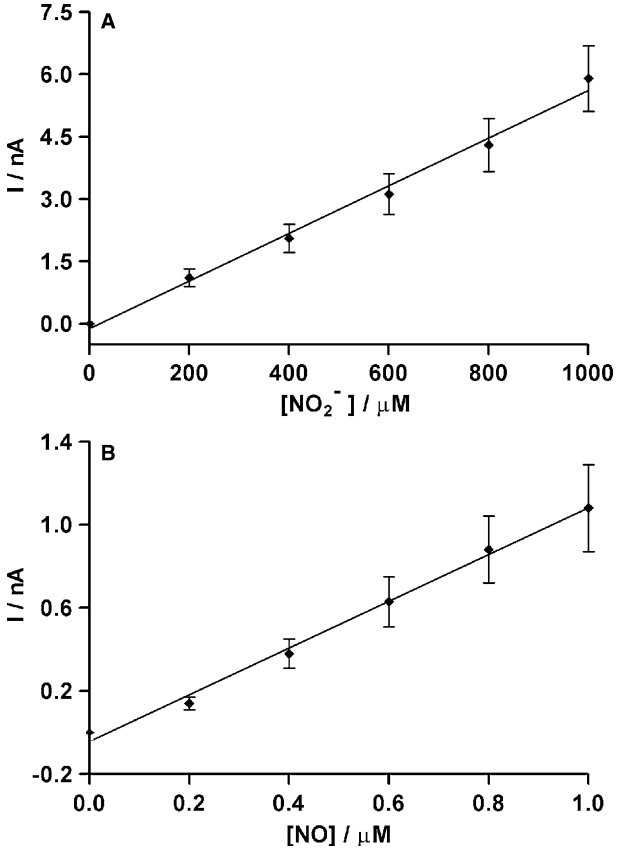

Fig. 5 **A** Amperometric calibration plot for NO_2^- in the concentration range 200–1000 μmol L^{-1} determined in PBS, pH 7.4, at +0.9 V (versus SCE) at Pt-disk electrodes ($n = 4$). **B** Amperometric calibration plot for NO in the concentration range 0.2–1.0 μmol L^{-1} determined in PBS, pH 7.4, at +0.9 V (versus SCE) at Pt-disk electrodes ($n = 28$)

Although, using UV–visible spectroscopy, it is possible to further investigate the possibility of NO_3^- contaminating the NO stock solution by the reduction of NO_3^- using a Cu-coated cadmium reagent [47], it was not considered necessary, because NO_3^- is not electroactive. Also, Zhang et al. [35] have previously reported that NO reacts with O_2 to produce, primarily, NO_2^- with little or no NO_3^- formation.

The fact that the method of NO synthesis described in this manuscript does not produce an NO stock concentration of 1.9 mmol L^{-1} at room temperature, is not inconsistent with other NO stock solutions previously used for experimental purposes. Cserey and Gratzl [45] have reported a stock NO concentration of 2.4 ± 0.3 mmol L^{-1} at room temperature which was generated by bubbling buffer with chemically generated NO obtained by reacting KNO_2 with excess ascorbic acid at a pH lower than 3. Lantoine et al. [48] have previously worked with NO stock solutions with a concentration of 1.4 mmol L^{-1} at room temperature. Their stock NO solution was produced by bubbling PBS with pure NO gas (Aldrich Chemical Co., St Louis, MO). Mas et al. [53] have used a stock NO solution with a concentration of between 0.17 and 0.2 mmol L^{-1} for characterisation and calibration of their electrochemical NO sensor. The NO stock solution was prepared by bubbling deoxygenated PBS with commercial NO gas (Air Liquide, Paris, France). Huang et al. [54] also prepared NO stock solutions from commercial NO gas. They analysed their NO stock solution using a commercial NO analyser (Sievers Instruments, CO, USA) and found their NO stock concentration to vary from 1.2 to 2 mmol L^{-1} at room temperature.

Stability of NO stock solution

Finally, because of the high reactivity and known instability of NO, in order to be able to perform accurate and reliable sensor calibrations it was decided to test the stability of the NO stock solution over time. The stability of the NO stock solution was thus monitored

Table 1 Time course of the concentration changes observed in the NO stock solution (1:20 dilution) over a 2-h period

Time (min)	$[NO]_{stock}$ (mmol L^{-1})
0	0.454
10	0.442
20	0.438
30	0.426
40	0.406
50	0.392
60	0.376
70	0.362
80	0.301
90	0.294
100	0.262
110	0.256
120	0.240

for up to 2 h after synthesis (Table 1). Very little change (ca. 6%) was observed in the stock NO concentration over the first 30 min. However, a much larger and more significant decrease was observed after 120 min, with the NO stock concentration decreasing by ca. 47% to 0.214 mmol L^{-1}. All NO calibrations were thus completed within 30 min of production of freshly prepared NO stock.

Conclusions

We have refined and developed a method of NO synthesis, using neutral Griess reagent, which has a gas collection time of 15 min, is inexpensive, simple to perform, and provides a reliable method of generating NO gas for in-vivo sensor calibration. The concentration of the generated NO stock solution was determined using UV–visible spectroscopy and found to be significantly less than the literature-quoted value for a saturated solution. The level of NO_2^- contaminant, also determined using spectroscopy, was not sufficient to cause any considerable increase in oxidation current when the NO stock solution is used for electrochemical sensor (bare Pt-disk electrodes) calibration over physiologically relevant concentrations. The NO stock solution was found to be stable for up to 30 min after synthesis and all sensor calibrations should be performed within this period.

As outlined in the Introduction, we have recently performed a detailed systematic study of various Nafion coating and heat curing procedures in order to design a simple and reproducible polymerisation method to maximise permselective characteristics, and thus eliminate signals from electroactive interferents, in sensors designed for direct in-vivo neurochemical measurements [12]. A novel pre-coating and curing method results in a device which has no interference from endogenous interferents such as ascorbic acid. Future work will focus on investigating the application of this technology in the development of a reliable, interference-free microelectrochemical sensor for measurement of brain extracellular NO. The NO synthesis method described in this manuscript will be used to generate stock solutions for sensor calibration.

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