



NO Microsensors

Microsensors for Nitric Oxide Measurement



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INSTRUCTION MANUAL

WPI Order No. _____

100505

World Precision Instruments

Warranty

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

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- Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department for a request form.
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- Equipment which was built as a special order cannot be returned.
- Always refer to the RMA# when contacting WPI to obtain a status of your returned item.
- For any other issues regarding a claim or return, please contact the RMA department.

Warning: This equipment is not designed or intended for use on humans.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

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Nitric Oxide Microsensor Guide

All WPI nitric oxide microsensors are 100% compatible with the original ISO-NO, the more recent ISO-NO Mark II (NOMK2) and the new APOLLO 4000 Free Radical Analyzer.

APPLICATIONS	<i>in vivo</i>	Cell Cultures	Cell Cultures, NO ₂ , NO ₃ ⁻	Tissue Bath	Tissue Bath	Microvessels	Microvessels	Single Cell
SENSOR	ISO-NOPF	ISO-NOPMC	ISO-NOP	ISO-NOP30L	ISO-NOP70L	ISO-NOP30	ISO-NOP007	ISO-NOPMM
SENSOR DIAMETER	100 & 200 µm	4 mm	2 mm	30 micron	70 micron	30 micron	7 micron	100 nm
RESPONSE TIME (with NOMK2)	< 5 sec	< 5 sec	< 5 sec	< 3 sec	< 3 sec	< 3 sec	< 3 sec	< 3 sec
LOWEST DETECTION LIMIT	0.2 nM	0.3 nM	1 nM	1 nM	1 nM	1 nM	0.5 nM	0.5 nM
TEMPERATURE SENSITIVITY	some	some	yes	yes	yes	yes	yes	some
DRIFT	none	none	none	none	none	none	none	none
SENSITIVITY	10 pA/nM	15 pA/nM	2 pA/nM	1.4 pA/nM	1.4 pA/nM	1.4 pA/nM	1 pA/nM	0.5 pA/nM
PHYSIOLOGICAL INTERFERENCE	none	none	none	none	none	none	none	none

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2. "A new nitric oxide gas sensor based on reticulated vitreous carbon/naion and its application" Jie Sun, Peter Huser, Valentin Zhelyaskov, Jie Lin, Mark Broderick, Harry Fein, Xueji Zhang, *Electroanalysis*. 2004,16, 1723-172.
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7. "An integrated nitric oxide sensor based on carbon fiber electrode coated with selective membranes" Xueji Zhang, Levis Cardosa, Mark Broderick, Harry Fein, Jie Lin, *Electroanalysis*. 2000,12(14), 1113-1117.
8. "A novel method to calibrate nitric oxide microsensors by stoichiometrical generation of nitric oxide from SNAP" Xueji Zhang, Levis Cardosa, Mark Broderick, Harry Fein, Ian R. Davis, *Electroanalysis*. 2000, 12(6),425-428.

7. Purge the system with NO for 5-10 minutes if using a pure source (longer if the NO source is not pure). Again make sure that gas is bubbling through both solutions. **Warning: NO is now escaping from the pressure relief needle in the stopper of the distilled water vial. It is imperative that the fume hood be running at maximum capacity with the front panel closed (in the down position).**
 8. After the time in step 7 has elapsed turn off the NO source.
 9. Immediately remove the two needles from the distilled water vial.
 10. Set the tee purge valve for purging with argon (or nitrogen) gas, and turn on the argon source. Purge the system for 5-10 minutes at a moderate flow rate. Gas should be bubbling through the KOH and then escaping from the flask into the atmosphere. Again be sure that the fume hood is ventilating well.
 11. Turn off the argon (or nitrogen) source, and allow the fume hood to continue to ventilate for 10-15 minutes so as to ensure that all traces of NO gas are removed from the atmosphere.
 12. The solution of distilled water should now be saturated with NO. The concentration of NO produced by this saturation is dependent upon the temperature. At 0° C, the concentration is approximately 3.3 mM, and at 20°C the concentration is approximately 1.91 mM.
 13. Dilutions of known concentration can be prepared from this saturated solution In preparing a dilution, **be careful not to unseal the vial**, for this exposes the solution to atmospheric oxygen.
- Once the dilutions are prepared, it is a simple matter to calibrate the instrument.
- source, and switch the tee purge valve to the correct position for purging with NO from the lecture bottle.

which the tubing passes by means of a Luer fitting to a syringe needle which extends almost to the bottom of the flask. Tubing is used to connect the side arm of the flask to the vial containing the water to be equilibrated with NO. The KOH solution is used to remove other nitrogen oxides from the NO gas.

- Place 20 mL of distilled (preferably deionized) water in a small glass vial. Seal the vial with a stopper and insert through the stopper a long syringe needle which extends almost to the base of the vial. Connect this syringe needle to the tubing from the KOH flask as illustrated. Insert an additional shorter syringe needle which **should not extend into the solution**. This acts as a pressure relief during purging.
- Place the distilled water vial in an ice-water bath. Reducing the temperature increases the solubility of NO in solution. Thus when the solution is used at room temperature you will be assured of a saturated NO solution.
- Purge the system with argon (or nitrogen) gas for a period of 30 minutes at a moderate flow rate such that the pressure is maintained at a safe level (1-2 psi). When purging it should be observed that gas is indeed bubbling through the KOH solution as well as the distilled water. After 30 minutes turn off the argon

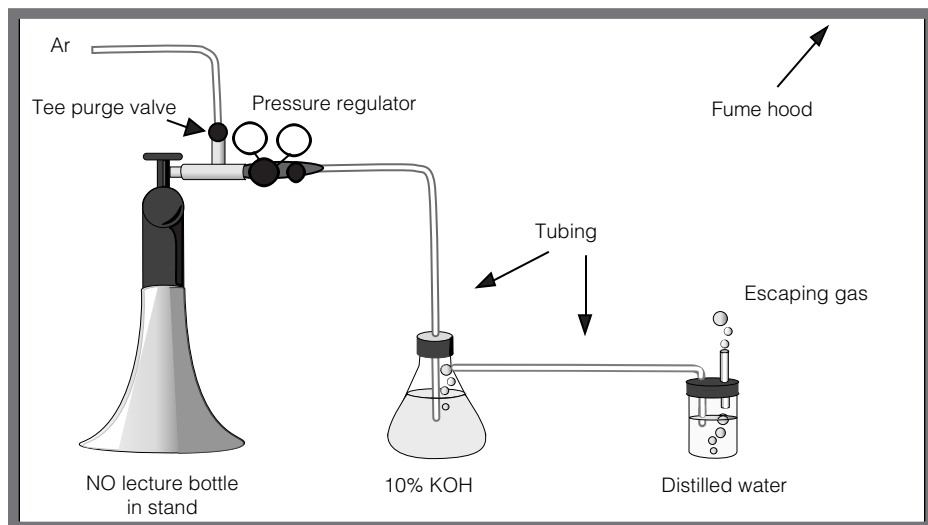


Fig. 5 — Setup for preparing a saturated NO aqueous solution must be in a fume hood with forced ventilation. Nitric oxide is highly toxic and, as illustrated, escapes into the atmosphere during preparation of the standards. Lecture bottle of NO (14.2 liters, 98.5%) obtained from Aldrich, catalog #29.556-6; telephone 800-558-9160

Unpacking the Nitric Oxide Microsensor

Due to the microsensor's extremely small size and intricate construction, great caution should be used when handling it. Avoid bringing the sensor tip into contact with solid surfaces, since this may break it. The sensors are packaged in foam holders so that their tips are physically isolated from contact with the container. To remove a microsensor from the package, use the thumb and index finger of one hand to separate the slit in the foam which contains the sensor and use the thumb and index finger of the other hand to grasp the sensor at its midsection and gently remove it from the container.

Attaching the Microsensor to the Microprobe Handle

Once removed from the package, the microsensor should be plugged into the microprobe handle which is connected to the **Apollo 4000** or **Apollo 1000**, again being very careful that the sensor tip does not come into contact with anything which could damage it. The sensor should plug in easily. If you encounter resistance, it is probably due to misalignment of the sensor plug with the socket connector inside the microprobe handle. Simply realign the sensor by gently rotating it until it snaps into place.

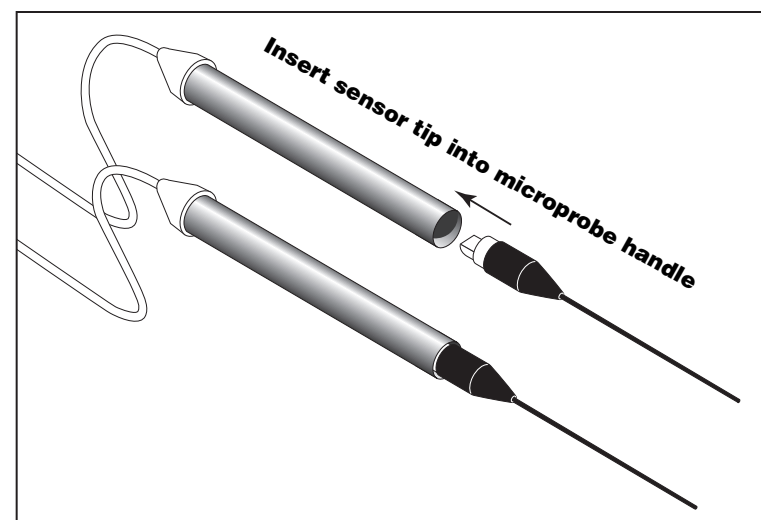


Fig. 1 — WPI NO microsensors offer unprecedented performance in making real-time in situ measurements — thanks to proprietary selective coatings and unique construction, combining both working and reference electrode as well as electrostatic shielding into a single sensor structure.

When a non-polarized microsensor is initially connected to an **Apollo 4000** or **Apollo 1000**, it may display a high (sometimes off-scale) background current. The polarization voltage applied by the instrument will cause a reduction of the background current to a stabilized baseline value over time. The amount of time required to reach a stable baseline current varies for each sensor. New sensors typically take longer, on the order of several hours. Once a stable baseline current is achieved (usually less than 2 nA), the microsensor is ready for use.

Using the Nitric Oxide Microsensor

First, calibrate the sensor using the NO calibration kit included with the Apollo 4000 or Apollo 1000. (The calibration kit, part #15829, may also be purchased separately.) See page 4 for instructions on calibrating the sensor.

Calibration of NO sensor using aqueous standards prepared with NO Gas

The following method can be used with all NO sensors.

WARNING: Nitric oxide must be handled only in a well-ventilated area, usually a laboratory fume hood with forced ventilation. The U.S. Occupational Safety and Health Administration has set a time-weighted average maximum NO value as 25 ppm. That is to say that 25 ppm is cited as the maximum concentration to which workers may be continually exposed. Brief inhalation of concentrations as low as 200 ppm could produce delayed pulmonary edema which may be fatal after an asymptomatic period of up to 48 hours after the initial exposure. It is therefore critical that the personnel handling the gas be thoroughly familiar with the Material Safety Data Sheet (MSDS) and proper handling procedures. The precautions recommended by the gas manufacturer must be followed.

Preparing an NO Standard

This method has the advantage of allowing the user to calibrate ISO-NO in the same environment in which the experimental measurements will be made. It has the disadvantages of added cost, inconvenience, and greater hazard to the user. All of these factors must be taken into consideration. The setup for preparing a saturated NO aqueous solution is illustrated in Figure 5.

1. **Be certain the fume hood is functioning.** See figure 5.
2. Make sure that all fittings and connections are secure. The tubing to be used should not be permeable to NO. We recommend Tygon® tubing if a polymer tubing is to be used; this is permeable to NO but has the best performance compared to other polymer tubing of which we are currently aware. Ideally glass tubing should be used. If Tygon® tubing is used, note that prolonged exposure to NO affects its properties; therefore it is recommended that the tubing be inspected frequently and that it be replaced when it appears to be brittle. The pressure regulator and tee purge adaptor should be stainless steel. This is because nitric oxide is a corrosive gas.
3. Prepare 100 mL of a 10 % (by weight) KOH solution and place it in the sidearm flask as illustrated above. The flask should be sealed with a stopper through

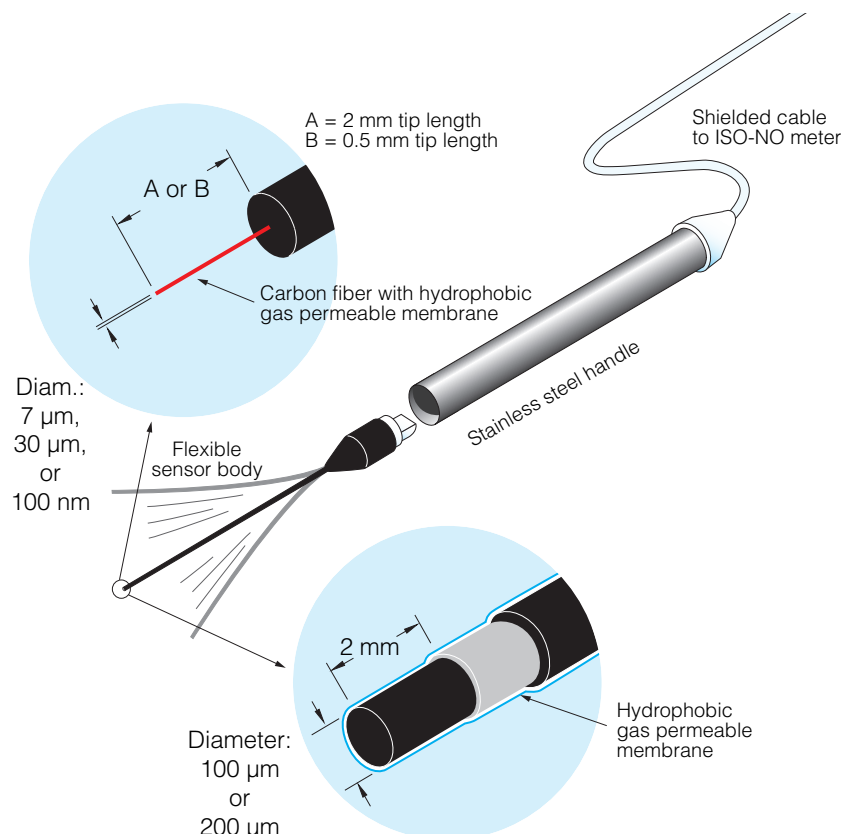


Fig. 2 — WPI NO microsensors may be changed or replaced quickly and easily.

liberated under the proposed set of parameters. It is assumed the other 40% of SNAP is either not decomposed or a proportion that is decomposed to NO is subsequently oxidized immediately before it is detected by the NO sensor.

Example for creating a calibration curve and related computations

1. SNAP weight = 6.4 mg.
2. SNAP was dissolved in 250 mL solution #1 to obtain the standard stock solution.
3. 20 μ L, 40 μ L, and 80 μ L of SNAP stock were added sequentially into 10 mL of solution # 2.
4. The current was continuously recorded during the course of the calibration.
5. A standard calibration curve was constructed according to the recorded data.

[SNAP]	[NO] = 0.6 X [SNAP]	Output Current
232.4 nM	139.4 nM	230 pA
462.0 nM	277.2 nM	488 pA
916.8 nM	550.1 nM	1001 pA

$$Y = -32.038 + 1.88X$$

The data from calibration curve indicates that this procedure allows an excellent linear calibration of NO probes ($R^2= 1$). The accuracy of calibration is approximately +/- 10% from mean. The source of error arises most probably from gravimetric measurement of the standard reagent, SNAP. In addition, purity of SNAP as well as partial oxidation of generated NO in the calibration solution could contribute to this error. Such a deviation may not be so important when NO is quantified in biological systems because most often the ability to measure changes in the basal concentration of NO is more significant than measurement of the absolute level of NO.

After completing the calibration, you may place the sensor in the experimental set-up and commence NO monitoring as explained in the Apollo instruction manual.

Storage and maintenance of NO microsensors

When not being used for a short period of time (such as overnight), the microsensor should remain attached to the microprobe handle and kept dry (not immersed in solution). Before the next experiment, immerse the sensor in the experimental solution (e.g., Krebs's Buffer); the background current will increase until reaching a stable value. Do not be alarmed if the background current becomes elevated. This is associated with the hydration of the sensor and will not negatively affect the sensor's performance.

If the microsensor is to be stored for a long period of time then it may be stored dry by removing it from the microprobe handle and returning it to the case in which it was shipped, being very careful to avoid making contact with the sensor tip.

The NO microsensor is a maintenance free consumable sensor: when it no longer functions, remove it from the microprobe handle and dispose of it, replacing it with a new one.

Calibration of NO microsensor by decomposition of SNAP

This method can be used to calibrate all NO sensors (see Ref. 1: Zhang, *et al.*, "Novel Calibration Method for Nitric Oxide Microsensors by Stoichiometrical Generation of Nitric Oxide from SNAP" *Electroanalysis*, 2000, **12**: 6).

S-nitroso-N-acetyl -D,L-penicillamine (**SNAP**) is a stable NO containing compound that can be used for quantitative generation of NO in solution. SNAP decomposes to NO and a disulfide by product when dissolved in water. However, the rate of decomposition of the SNAP is very slow. The kinetics controlling the decomposition of SNAP depends on several parameters including, pH, presence of catalyst, temperature and light [2,92]

In the procedure described here, SNAP is used in combination with a catalyst, cuprous chloride, to generate known amounts NO in solution, which can then be used to accurately calibrate various NO-sensors. The protocol does not investigate all parameters involved in SNAP decomposition neither is it intended to propose a model by which SNAP is decomposed.

Calibration by decomposition of a S-nitrosothiol compound using Cu(I) as a catalyst: Method 1

CAUTION: The described calibration procedure requires the use of cuprous (I) chloride, CuCl, where Cu (I) is the active catalyst for the conversion of SNAP to NO. The calibration curve assumes only the presence of Cu (I) and hence a 100% conversion efficiency of SNAP to NO (see "A novel method to calibrate nitric oxide microsensors by stoichiometrical generation of nitric oxide from SNAP", X. Zhang, L. Cardosa, M. Broderick, H. Fein, I. R. Davis, *Electroanalysis*, 2000, 12(6),425-428). However, in the presence of oxygen Cu (I) is readily oxidized to Cu (II). This will happen naturally if the compound is exposed to air and/or there is inadequate storage of CuCl. The oxidation product Cu (II) is much less efficient at catalyzing the conversion of SNAP to NO, and this would appear during calibration as an apparent low sensitivity of the electrode to NO.

Since Cu (I) is readily oxidized to Cu (II) special precautions must be taken to keep it in its reduced state prior to any calibration. It is recommended that CuCl be stored under inert conditions and if used in solution then the solution must be degassed with inert gas and absent of all oxygen.

NOTE: If your laboratory is not adequately equipped to satisfy the conditions for

Note: Remember that most NO probes are sensitive to changes in temperature. It is therefore recommended that the calibration of your sensor is performed at experimental temperature.

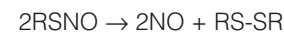
Predicting the Level of Detectable NO According to the Molar Ratio of SNAP in the Presence of Catalyst

A calibrated NO sensor was used in a series of experiments to measure SNAP decomposition in the presence of copper sulfate. The data from these experiments indicated that SNAP is decomposed instantaneously under the following set of experimental conditions:

Temperature	25°C
Catalyst solution	0.1M copper sulfate or copper chloride
SNAP	WPI, 98% purity. Fresh stock solution with 5 mg/250 mL solution EDTA added.

Copper sulfate is at equilibrium with ambient air (aerobic conditions).

SNAP (RSNO) decomposes to NO and a disulfide byproduct according to the following equation:



Theoretically, the concentration of generated NO should be equal to the final concentration of SNAP in the copper sulfate solution in the calibration vial if the decomposition goes to completion and if the generated NO is detected quickly before it is oxidized to nitrite and nitrate.

However, it is expected that the level of detectable NO will be below the theoretical value because the copper sulfate solution was at equilibrium with ambient air, and consequently a portion of the generated NO would have been immediately oxidized to nitrite and nitrate before it was measured by the NO sensor. In addition, it is possible that decomposition of SNAP does not go to completion even in the presence of a catalyst. Results on the kinetics of SNAP decomposition in the presence of a catalyst in an anaerobic environment are published elsewhere (Zhang *et al.*, "Novel Calibration Method for Nitric Oxide Microsensors by Stoichiometrical Generation of Nitric Oxide from SNAP", *Electroanalysis*, 2000, **12**: 6).

Our experimental data indicates a conversion efficiency of SNAP to NO of approximately 0.6 (*i.e.*, 60%). This result is only applicable for calibration of a NO sensor in a solution, which is at equilibrium with ambient air and at the experimental conditions described above. Hence for each mole of SNAP, 0.6 mole of NO is

and the stock solution of SNAP remains relatively stable for at least 5 hours if kept in refrigerator.

Note: The purity of standard reagent, SNAP, is very important for the reported data. Use high grade SNAP with minimal purity of 95% or better. SNAP can be purchased from WPI (catalog # **SNAP50**, **SNAP100**, **SNAP500**).

Calibrating a probe

Place 10.0 mL of solution #2 in a 20 mL vial (supplied in the calibration kit). Drop a small stirring bar into the solution, and place the vial on the top of a magnetic stirring plate. Immerse a NO probe into this solution, and while stirring, allow the background current to stabilize for about 3-5 minutes. As soon as the background current becomes stable start the recording.

Next, sequentially inject three aliquots of SNAP solution, 5 μL , 10 μL , and 20 μL , into the vial containing copper sulfate solution. The current output will rapidly increase upon addition of first aliquot and will reach a plateau within a few seconds. Inject the second aliquot, 10 μL , as soon as the first signal reaches a plateau. Finally add the third aliquot as the second signal reaches its plateau. If aliquots are not added promptly when reaching the previous plateau, the signal will slowly decline because generated NO is quickly oxidized to nitrite and nitrate which will not be detected by the probe.

Note: You can change the volume of injected aliquots according to the concentration of SNAP stock solution. Decrease the volume of aliquot if the electrode is very sensitive or increase the volume of aliquot if the electrode is less sensitive.

Because NO sensors can be calibrated in a linear fashion, the magnitude of every signal should almost double as the volume of SNAP solution added is doubled in the course of the calibration. Use the recorded data to construct a calibration curve. The calibration curve can be simply constructed by plotting the signal output (*e.g.*, in pA) vs. the concentration of SNAP added at that time. Note that every addition of SNAP solution corresponds to a particular NO concentration. This will be discussed below. After the sensitivity of the NO probe is established, the meter (Apollo 4000 or Apollo 1000) can be calibrated to display data in either concentration mode (*i.e.*, nM, mM) or redox current (*i.e.*, pA, nA).

The standard SNAP solution can be used for the calibration of NO probes throughout the day. Store the solution in the dark and refrigerate when not in use. Prepare a fresh stock solution of SNAP in the beginning of every day to ensure minimal decomposition of SNAP in the stock solution. Concentration of SNAP decreases to 5-10% of its nominal value after approximately 4-5 hours.

storage and use of CuCl please refer to the following section in the manual which describes a similar calibration procedure based on the use of copper (II) sulfate (CuSO_4) or cupric (II) chloride CuCl_2 , in which Cu [I] is the active catalyst for the conversion of SNAP to NO.

Getting Started

Prepare the following solutions:

#1—Saturated solution of cuprous chloride:

This should be prepared by adding 150 mg CuCl to 500 mL distilled deoxygenated water. The distilled water can be deoxygenated by purging with pure nitrogen or argon gas for 15 min. The saturated CuCl solution will have a concentration of approximately 2.4 mM at room temperature and should be kept in the dark prior to use.

#2—Standard SNAP solution:

Dissolve 5 mg EDTA in 250 mL of HPLC pure water (HPLC grade, Sigma). Deoxygenate the solution using the method described above. Add 5.6 mg SNAP to the solution. The Molarity of the SNAP solution (SNAP f.w.= 220.3) can then be easily calculated. Since the SNAP solution is very sensitive to light and temperature it should be stored in the dark and in a refrigerator until required. (**Note:** The decomposition of SNAP at low temperature, in dark and in absence of trace metal ions proceeds very slowly due to the presence of chelating reagent, EDTA). The standard SNAP solution can then be used for many calibrations of NO probes throughout the day. However, since SNAP will slowly decompose even if stored correctly as described, it is recommended that a fresh standard stock solution of SNAP is prepared at the beginning of everyday. This will ensure an accurate calibration of the NO sensor.

The concentration of SNAP in the stock solution is calculated as follows:

$$[C] = [A \cdot W / (M \cdot V)] 1000$$

Where C is the concentration of SNAP in micromolar (μM), A is the purity of SNAP, W is weight of SNAP in milligram (mg) and V is the volume of the solution in liter (l). If SNAP purity is 98.5%, hence in the above example the concentration of SNAP is:

$$[C] = [98.5\% \times 5.6 / (220.3 \times 0.25)] \times 1000 = 100.1 \mu\text{M}$$

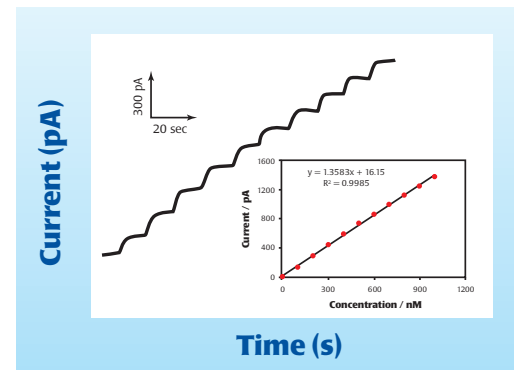


Fig. 3 — Responses to the successive additions of 100 nM SNAP into CuCl saturated solution (pH=5.5). Also shown (inset) is the resulting calibration plot.

Note: The purity of SNAP used is extremely important to ensure an accurate calibration. We recommend the use of high grade SNAP with minimal purity of 98% or better. SNAP can be purchased from WPI in various amounts.

Calibration of the NO probe

Within a nitrogen or argon environment, place 10.0 mL of solution #1 (CuCl) in a 20 mL vial (supplied in the calibration kit). Drop a small stirring bar into the solution, and place the vial on the top of a magnetic stirring plate. Immerse a NO probe into this solution, and while stirring, allow the background current to decay and stabilize for 3-5 min. As soon as the background current becomes stable, start recording the data from the Apollo 4000. We recommend a computer based data-acquisition system, such as WPI's **Lab-Trax** when using Apollo 1000.

Next, inject 3 aliquots containing 5 μ L, 10 μ L and 20 μ L sequentially of the SNAP stock solution into the vial containing cuprous chloride solution. Depending on the required calibration range (*i.e.*, the final amount of NO produced) desired, the volumes of SNAP stock solution could be increased to produce a greater concentration of NO. It is recommended that calibration range be kept close to the anticipated experimental concentration of NO.

Immediately following the first addition of SNAP into Solution#1 the current (pA) output from the Apollo will be seen to increase rapidly. Within a few seconds the response will reach a plateau and the second aliquot of SNAP can then be added. Successive additions of the remaining aliquots of SNAP can be made in a similar way.

Note: If aliquots of SNAP are not added promptly when reaching the previous plateau, the output signal will be seen to slowly decline as the NO generated is oxidized to nitrite and nitrate, which are not detected by the NO sensor).

A calibration curve can be constructed by plotting the signal output (pA) vs concentration (nM) of SNAP. Each addition of SNAP corresponds to equivalent NO concentration. The response should be very linear from 10 to 1000 nM.

The sensitivity of the NO probe can be established from the gradient of the response curve. The sensitivity of the microsensors is 1-100 pA/nM. Once the slope of the probe has been determined the value can be entered into the Apollo software if the user wishes the observe data in concentration mode (*i.e.*, nM, mM). However, most users prefer to use the Apollo in the default redox current mode (*i.e.*, pA, nA).

Note: Remember that most NO probes are sensitive to temperature changes. It is therefore recommended that the calibration of a NO sensor is performed at the experimental temperature.

Calibration by decomposition of SNAP using Cu(II) as a catalyst: Method 2

The calibration procedure described here can be used as an alternative to the previous method in which Cu (I) is the active catalyst for the conversion of SNAP to NO. In this procedure Cu[II] is substituted as a catalyst for ease-of-handling.

Note: Experimentally it has been show that Cu[II] is less efficient as a catalyst in the conversion of SNAP to NO (*e.g.*, conversion ratio is reduced to approximately 60%). The accuracy of the calibration may also be reduced (see discussion).

S-Nitroso-N-acetyl-D,L-penicillamine (SNAP) is a stable NO containing compound that can be used for quantitative generation of NO in solution. SNAP decomposes to NO and a disulfide byproduct when dissolved in water. However, the rate of decomposition is very slow. The kinetics of decomposition for this reagent is a function of several parameters including pH, presence of a catalyst, temperature and light.

In the procedure described here, SNAP is used in combination with a catalyst, cupric (II) sulfate (CuSO_4) or cupric (II) chloride (CuCl_2), to generate a known quantity of NO in solution. Note that this protocol does not investigate the effects of all parameters involved in SNAP decomposition nor does it propose a model by which NO is decomposed. The presented procedure provides an empirical estimation of the amount of generated NO based on the molarity of a standard stock solution of SNAP under a controlled set of parameters.

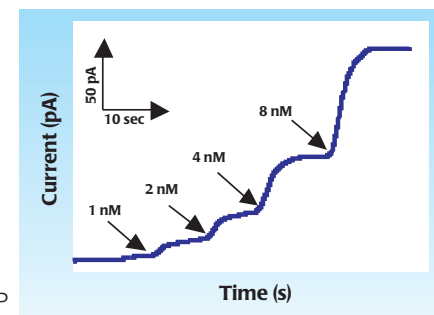


Fig. 4 — Response of ISO-NOPF to NO.

Getting Started

Prepare the following solutions:

Solution #1: Dissolve 5 mg EDTA in 250 mL of water (HPLC grade).

Solution #2: Prepare 250 mL 0.1 M copper (II) sulfate (or cupric (II) chloride) in distilled water.

Preparing standard SNAP solution

To prepare the stock solution of SNAP, weigh approximately 5.0 mg \pm 2.0 mg of SNAP and add it to solution #1. Calculate the Molarity of SNAP solution (SNAP f.w. = 220.3). Decomposition of SNAP in the stock solution proceeds very slowly due to the presence of chelating reagent, EDTA. Thus the rate of decomposition is negligible