Liquid NO application

The CLD 88 enables to detect down to pMol level (nitrite / nitrate levels in drinking water).
The new sample tube we are using (type 1), is optimized for this application. The internal flow restriction is made from stainless steel and it can be cleaned in an ultrasonic cleaner. The sample flow of 110 ml/min minimizes the effect of the helium bubbles in the liquid sampler, which results in very low noise signal in the NO analyzer.

Liquid Applications

Several techniques have been developed for the measurement of NO and its oxidation products using the Liquid Purge Vessel system in conjunction with the CLD 88 (Nitric Oxide Analyzer). These techniques include:

- Reduction of nitrate using vanadium(III) and hydrochloric acid at 90 °C.
- Reduction of nitrite using iodide and acetic acid
- Reduction of nitrosothiols using a modified Saville reaction or Cu/Cystine.

Nitrates

Nitrate (NO_3^-), the major oxidation product of NO in some cell culture systems and most physiological fluids, is formed when nitric oxide reacts with oxyhemoglobin or the superoxide anion. To measure nitrate, vanadium(III) chloride in hydrochloric acid is used to convert nitrate to nitric oxide.

Vanadium(III)/HCl will also convert nitrite and S-nitroso compounds to NO.

$$2NO_3^- + 3V^{3+} + 2H_2O \rightarrow 2NO + 3VO_2^+ + 4H^+$$

To achieve high conversion efficiency, the reduction is performed at 90 °C. For most applications, ~5 mls of the reducing agent is prepared in the purge vessel. That volume is usually sufficient for measurement of 20 - 50 samples, depending on the volume of sample injected. To prevent damage to the CLD 88 from the hydrochloric acid vapor, a gas bubbler filled with aqueous sodium hydroxide is installed between the purge vessel and the CLD 88.[1]

Nitrites

Nitrite (NO_2^-), the major oxidation product of NO in the absence of oxyhemoglobin or superoxide anion, is formed when nitric oxide reacts with dissolved oxygen. To measure nitrite in cell culture systems, perfusates and other liquid samples, the purge vessel contains the reducing agent potassium iodide in acetic acid to convert nitrite to nitric oxide.

$$I^- + NO_2^- + 2H^+ \rightarrow NO + \tfrac{1}{2}I_2 + H_2O$$

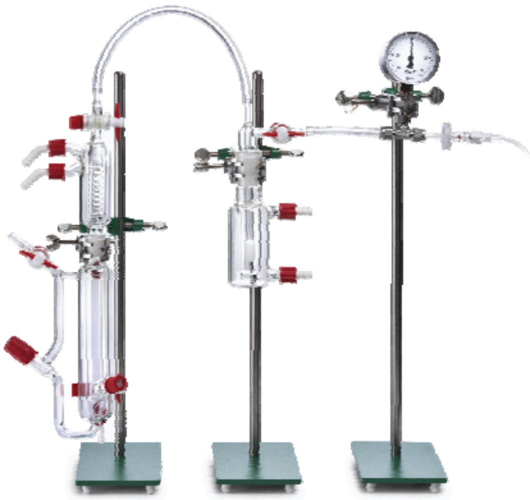
For most applications, ~5 mls of the reducing agent is prepared in the purge vessel. That volume is usually sufficient for measurement of 20 - 50 samples, depending on the volume of sample injected. The reaction is performed at room temperature.[2]

Nitrosothiols

Nitrosothyols are important reaction products of NO and can also be measured. A modified Saville reaction is suggested for measurement of high molecular weight species such as S-nitrosoalbumin or hemoglobin. To perform this procedure, it is first necessary to measure the concentration of nitrite (NO_2^-) using I^- / acetic acid. Next, add excess $HgCl_2$ and measure the concentration of NO_2^- again. The [RSNO] can be easily calculated from the difference of the [NO_2^-] before and after. For lower molecular compounds like S-nitrosoglutathione, the Cu(I)/Cysteine reaction is recommended. This reduction is performed using a saturated solution of CuCl in PBS with 100 μMol cystine. The technique is selective for RSNO's. Standards are prepared by the reaction of thiol with nitrite.[3]

Liquid Accessories

The Liquid Purge Vessel, used in conjunction with the CLD 88 and chemical reducing agents discussed above, will enable rapid reaction products of all reaction products of nitric oxide. Chemical reducing agents are used, not slow reacting enzymatic agents, to convert the reaction products to Nitric Oxide. The purge vessel's design ensures quantitative conversion of nitrate/ nitrite/ nitrosothiols to nitric oxide, resulting in sharp, well-defined data peaks. Clean up is quick and simple with the built-in drain port.



Data Analysis

The PowerChrom software from EDAQ enables easy calibration and flexible chromatographic data analysis.

1. Braman, et al., *Anal. Chem.* 1989; 61: 2715-8
2. Castegnaro, et al., *Food Addit. Contam.* 1978, 4:37-43
3. Fang, et al., *Biochem Biophys Res Comm* 1999;252:535-540.