Pharmaceutica Analytica Acta
ISSN: 2153-2435

Shintani, Pharm Anal Acta 2013, 4:6
DOI: 10.4172/2153-2435.1000248

Short Communication Open Access

# ESR Spin Trapping

**Hideharu Shintani***

*Chuo University, School of Science, 1-13-27, Kasuga Bunkyo 112-0003 Tokyo, Japan*

**Keywords:** ESR; Spin-trapping; Nitric oxide; CO-hemoglobin; Blood

## Introduction

Election spin resonance (ESR) spectroscopy is a technique that can be applied to the detection and measurement of free radicals, because it detects the presence of unpaired electrons. An unpaired electron has a spin of either + or – and behaves as a small magnet. ESR spectroscopy is highly sensitive and can detect radicals at levels as low as $10^{-10}$ M, if they are sufficiently long-lived to be measured. For very unstable radicals in biological systems, however, alternative approaches, are required. One such example is spin-trapping, in which a highly reactive radical reacts with a trapping reagent producing a long-lived nitroxide radical, for example, that can be detected and measured [1].

## Protocol

1. Set ESR spectrometer as follows: Incident microwave power, 5-20 mW; modulation frequency, 100 kHz; modulation amplitude 0.3-1.0 Gauss; response time 0.5-1.0 s; sweep rate 12.5 Gauss/min.
2. Add the following spin trapping agent to the reaction mixture: 5,5-dimethyl-1-pyrroline N-oxide (DMPO); N-t-butyl-α-phenylnitrone (PBN); α-4-Pyridyl 1-oxide N-ttert-butyl nitrone (4-POBN).
3. Add the agent to start the reaction.
4. Place the reaction solution in an ESR quartz flat cell.
5. Place the flat cell in the cavity of the ESR spectrometer.
6. Adjust frequency control to get G dip at the center of display.
7. Adjust the flat cell to obtain a maximum G dip.
8. Adjust phase and iris.
9. Start the measurement, sequential ESR scans are then recorded.
10. Analyse the ESR signal of spin-trapping adduct.
11. To determine the spin concentration, integrate doubly the ESR signal and calculate the spin concentration by comparing the signal with that of a standard Tempol (2, 2, 6, 6-tetramethyl-4-hydroxypiperidine-1-oxyl) solution. The concentration of Tempol can be determined optically from the extinction coefficient at 240 nm of 1440 M/cm.

## Spin-Tapping of Nitric Oxide (NO) with CO-Hemoglobin (HbCO) in the Peritoneal Cavity

It takes a long time to displace oxygen from Hb. Because CO readily replaces oxygen in Hb and NO readily replaces CO in Hb, HbCO can be used as a trapping agent for NO. This method can be applied under low $pO_2$ [2].

## Protocol

1. Treat male Wistar rats (ca 100 g) intraperitoneally with nitric oxide synthetase-inducing agent, i.e. *E. coli* LPS (lipopolysaccharide).
2. Stir Hb solution in a closed flask and introduce CO gas (100%). After conversion of Hb to HbCO, remove excess CO gas in the HbCO solution by purging with nitrogen gas.
3. After induction of nitric oxide synthase, inject the HbCO solution into the peritoneal cavity.
4. A few hours later, aspirate peritoneal fluid by syringe, transfer to a quartz ESR tube through a long needle, and freeze immediately in liquid nitrogen. The syringe, long needle and the ESR tube should be prepared oxygen-free by means of flowing nitrogen.
5. Set the ESR spectrometer as follows: Incident microwave power, 5-20 mW; Modulation frequency, 100 kHz;

Modulation amplitude, 2-5 Gauss; response time, 0.-1.0 second sweep rate, 125 Gauss/min; temperature 77-150 K.

For stability and sensitivity, low-temperature operation is recommended. The cavity must be flushed with dry nitrogen gas during measurement.

6. Start the measurement after adjustment of the ESR spectrometer.
7. To see a three-line hyperfine structure characteristic of HbNO, the peritoneal fluid should be added in oxygen-free inositol hexaphosphate solution.

## Measurement of HbNO in Circulating Blood

HbNO in circulating blood can be detected, probably because of the low $pO_2$ of the venous circulation, and there is a spectral transition between A and V [3,4].

## Protocol

1. Treat rats with LPS or cytokines.
2. Collect blood (0.4 mL) from a vein or artery, transfer to an ESR tube through a long needle, and freeze immediately in liquid nitrogen.
3. Measure the ESR spectrum.
4. Determine HbNO concentration on the basis of double integration of the first derivative ESR spectrum, because of the spectral diversity of HbNO.
5. Prepare HbNO standard anaerobically with NO gas (*via* HbCO). Briefly, oxyHb is converted to HbCO. Then introduction of nitric oxide gas converts HbCO to HbNO.

***Corresponding author:** Hideharu Shintani, Chuo University, School of Science, 1-13-27, Kasuga Bunkyo 112-0003 Tokyo, Japan, Tel: +81425922336; E-mail: shintani@mail.hinocatv.ne.jp

**Received** May 20, 2013; **Accepted** June 12, 2013; **Published** June 15, 2013

**Citation:** Shintani H (2013) ESR Spin Trapping. Pharm Anal Acta 4: 248. doi:10.4172/2153-2435.1000248

**Copyright:** © 2013 Shintani H. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## References

1. Shimizu S, Eguchi Y, Kosaka H, Kamiike W, Matsuda H, et al. (1995) Prevention of hypoxia-induced cell death by Bcl-2 and Bcl-xL. Nature 374: 811-813.

2. Kosaka H, Shiga T (1996). Detection of nitric oxide by ESR using haemoglobin. In Methods in Nitric Oxide Research. Wiley and Sons 373-381.

3. Kosaka H, Sawai Y, Sakaguchi H, Kumura E, Harada N, et al. (1994) ESR spectral transition by arteriovenous cycle in nitric oxide hemoglobin of cytokine-treated rats. Am J Physiol 266: C1400-1405.

4. Kosaka H, Seiyama A (1997) Elevation of oxygen release by nitroglycerin without an increase in blood flow in the hepatic microcirculation. Nat Med 3: 456-459.