

# Electron spin resonance analysis of the oxidation reactions of nitron-type spin traps with gold(III) ion

Akira Nakajima,<sup>1,\*</sup> Yuto Ueda,<sup>2</sup> Nobuyuki Endoh,<sup>3</sup> Kunihiro Tajima,<sup>4</sup>  
and Keisuke Makino<sup>5</sup>

<sup>1</sup> Section of Chemistry, Department of Medical Science, Faculty of Medicine, University of Miyazaki, Kiyotake, Miyazaki 889-1692, Japan,

<sup>2</sup> Department of Psychiatry, Faculty of Medicine, University of Miyazaki, Kiyotake, Miyazaki 889-1692, Japan,

<sup>3</sup> The Wakasa Wan Energy Research Center, Nagatani 64-52-1, Tsuruga, Fukui 914-0192, Japan

<sup>4</sup> Department of Applied Biology, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan,

<sup>5</sup> International Innovation Center, Kyoto University, Yoshida, Sakyo-ku, Kyoto 611-8501, Japan

\* Corresponding author    Tel & Fax: +81-(0)985-85-1185.

*E-mail address : akanaka@med.miyazaki-u.ac.jp (A. Nakajima)*

**Abstract:** When cyclic nitrones, such as 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), 4-phenyl-5,5-dimethyl-1-pyrroline-N-oxide (PDMPO), and 3,3,5,5-tetramethyl-1-pyrroline-N-oxide (M4PO) were mixed with hydrogen tetrachloroaurate(III), DMPOX (5,5-dimethyl-1-pyrrolid-2-one-N-oxyl)-type free radicals appeared with the precipitation of Au(0). The reaction did not proceed with non-cyclic nitrones, such as N-*tert*-butyl- $\alpha$ -phenyl-nitron (PBN) and  $\alpha$ -(4-pyridyl-1-oxide)-N-*tert*-butyl-nitron (POBN). The order of the H<sub>2</sub>AuCl<sub>4</sub> decrease was DMPO > PDMPO > M4PO. The reaction was depressed by the addition of chloride or hydroxide ions. 1-Hydroxy-5,5-dimethyl-1-pyrrolid-2-one (HDMPN), the precursor of DMPOX, was also oxidized to DMPOX by H<sub>2</sub>AuCl<sub>4</sub>. Every step of gold reduction from Au(III) to Au(0) can be used for the oxidation of HDMPN to DMPOX. Based on these and previous results, the reaction was explained by the assumed scheme consisted of the ligand exchange interaction of AuCl<sub>4</sub><sup>-</sup> with >N<sup>+</sup>-O<sup>-</sup> in DMPO, the nucleophilic addition of water molecule to DMPO, the stepwise intra-molecular transfer of three electrons from DMPO to Au(III), and the precipitation of Au(0).

*Keywords* : ESR, nitron, spin traps, DMPO, DMPOX, gold(III) ion

**Résumé:** Lorsque des nitrones cycliques, tels que 5,5-diméthyl-1-pyrroline-N-oxyde (DMPO), 4-phényl-5,5-diméthyl-1-pyrroline-N-oxyde (PDMPO), et 3,3,5,5-tétraméthyl-1-pyrroline-N-oxyde (M4PO) ont été mélangés avec du tétrachloroaurate (III) d'hydrogène, des radicaux libres de type DMPOX (5,5-diméthyl-1-pyrrolidine-2-one-N-oxyl) apparut avec la précipitation de Au(0). La réaction ne s'est pas produite avec les nitrones non cycliques, tels que N-*tertio*-butyl- $\alpha$ -phényl-nitronne (PBN) et  $\alpha$ -(4-pyridyle-1-oxyde)-N-*tertio*-butyl-nitronne (POBN). L'ordre de la diminution de H<sub>2</sub>AuCl<sub>4</sub> était DMPO > PDMPO > M4PO. La réaction a été abaissée par l'addition d'ions chlorure ou hydroxyde. 1-Hydroxy-5,5-diméthyl-1-pyrrolidine-2-one (HDMPN), le précurseur de DMPOX, a été aussi oxydé en DMPOX par H<sub>2</sub>AuCl<sub>4</sub>. Chaque étape de la réduction de l'or de Au(III) à Au(0) peut être utilisée pour l'oxydation de HDMPN en DMPOX. Basée sur ceux-ci ainsi que sur les résultats précédents, la réaction a été expliquée par la combinaison supposée se composant de l'interaction d'échange du ligand de AuCl<sub>4</sub><sup>-</sup> avec >N<sup>+</sup>-O<sup>-</sup> dans DMPO, de l'addition nucléophile d'une molécule d'eau à DMPO, du transfert intramoléculaire par étape de trois électrons de DMPO à Au(III), et de la précipitation de Au(0).

## Introduction

Recently, various nitrones have been used as spin traps for short-lived free radicals, such as hydroxyl radical, in biosystems [1]. However, some of them are well known to produce free radicals through not only radical reaction but also ionic reaction.

5,5-Dimethyl-1-pyrroline-N-oxide (DMPO), one of the most popular nitron-type spin traps, was oxidized to 5,5-dimethyl-1-pyrrolid-2-one-N-oxyl (DMPOX) by various oxidants [2-11] or through oxidative bioreactions [12-17]. Similar DMPOX-type radicals were also observed for M4PO [9, 18, 19]. Makino *et al.* reported that DMPO was oxidized to DMPOX by ferric ion through 2-hydroxy-5,5-dimethyl-1-pyrrolidinyloxy (DMPO-OH), 2-hydroxy-5,5-dimethyl-1-pyrrolid-2-one (HDMPO), and 1-hydroxy-5,5-dimethyl-1-pyrrolid-2-one (HDMPN) [5]. Tajima *et al.* clarified in their electrochemical study that DMPO was oxidized to DMPOX by removal of three electrons [18,19]. As the use of various nitrones for spin trapping analysis has been increased recently, further information on specificities and kinetics of DMPOX-type radical formation for various nitrones should become very important.

Gold(III) ion has been well known to reduce to Au(0) by various compounds containing sulfur, such as methionine [20]. Recently, Zou *et al.* found that Au(III) oxidized glycine through deamination and decarboxylation with formation of glyoxylic acid, ammonium ion, formic acid, carbon dioxide and metallic gold [21]. These results suggest that Au(III) is one of the most excellent oxidants for various organic compounds, and can be applied for the analysis of the DMPO oxidation.

The authors, therefore, examined the interaction between Au(III) and nitron-type spin traps, such as DMPO, and found the formation of DMPOX-type free radicals. In this study, the aspects and the mechanism of the nitron oxidation reaction with Au(III) are discussed.

## Experimental

### Chemicals

Hydrogen tetrachloroaurate(III) (HAuCl<sub>4</sub>) dihydrate, and sodium dicyanoaurate(I) were obtained from Nacalai Tesque (Kyoto, Japan). 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO), 3,3,5,5-tetramethyl-1-pyrroline-N-oxide (M4PO), N-*tert*-butyl- $\alpha$ -phenyl-nitron (PBN), and  $\alpha$ -(4-pyridyl-1-oxide)-N-*tert*-butyl-nitron (POBN) were obtained from DOJINDO Ltd. (Kumamoto, Japan). During manufacture, greater than 99 % purity was obtained by repeated vacuum distillation and was checked by gas-chromatography.

4-Phenyl-5,5-dimethyl-1-pyrroline-N-oxide (PDMPO) was obtained from Sigma-Aldrich Japan Inc. (Tokyo, Japan). 1-Hydroxy-5,5-dimethyl-1-pyrrolid-2-one (HDMPN) was prepared according to the method described by Bonnett *et al.* [22]. Molecular structures of nitrones used in this study were shown in Fig. 1. All nitrones used and HDMPN were examined by ESR and no radical species was observed.

### Electron spin resonance measurements

Forty microliters of HAuCl<sub>4</sub> solution (1.25 mM) and 40  $\mu$ l of nitron solution (1.25 mM) were mixed at pH 4. After aspirating the mixture into a capillary tube, its ESR spectrum was immediately recorded using X-band ESR spectrometer (JES TE-100, JEOL Ltd., Tokyo, controlled by WIN-RAD ESR data analyzer, Radical Research Inc., Tokyo) under the following conditions: microwave power 5 mW; microwave frequency, 9.42 GHz; magnetic field, 335.3 mT; field amplitude,  $\pm$  5 mT; field modulation, 100 kHz; modulation width, 0.025 ~ 0.1 mT; sweep time 1 min; and the response time 0.1 s. The solution pH was adjusted to the desired value with 0.1 N HNO<sub>3</sub> and 0.1 N NaOH solutions. To avoid the photochemical

reduction of gold(III), reaction was conducted under dark condition. For the determination of radical concentration, 60  $\mu$ l of the sample solutions was aspirated into capillary tubes and their ESR spectra were recorded one by one. The concentration of DMPOX-type radical was calibrated by using TEMPOL as a standard, after double integration of observed ESR signal.

### **Gold determination**

Total amounts of gold(III) species in the reaction mixture was determined by spectrophotometry using Rhodamine B [23]. The amounts Au(0) resulted in the reaction mixture was determined as follows: the precipitate was dissolved in HNO<sub>3</sub>-HCl (1 : 3) solution (aqua regia), and then the concentration of gold in the solution was determined by the inductively coupled plasma quantometer (Shimadzu ICPQ-1000II).

### **Results**

#### **Formation of aminoxyl radicals in nitron-HAuCl<sub>4</sub> solutions**

When solutions of cyclic nitrones (1.25 mM), such as 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), 4-phenyl-5,5-dimethyl-1-pyrroline-N-oxide (PDMPO), and 3,3,5,5-tetramethyl-1-pyrroline-N-oxide (M4PO), were mixed with hydrogen tetrachloroaurate(III) (HAuCl<sub>4</sub>) solution (1.25 mM) at pH 4, ESR signals with 9, 10 and 3 lines appeared, respectively (Fig. 2). Its hyperfine coupling constants, being estimated from computer simulation, are listed in Table 1. From these values, the radicals were identified as 5,5-dimethyl-1-pyrrolid-2-one-N-oxyl (DMPOX) [5, 7, 12, 13] and 3,3,5,5-tetramethyl-1-pyrrolid-2-one-N-oxyl (M4POX) [9]. No  $\beta$ -proton in M4POX gave the primary nitroxide triplet without any additional hyperfine splitting. Radical species appeared in PDMPO-Au(III) solution has three hyperfine coupling constants, 0.71, 0.40 and 0.30 mT.

Two of them, 0.71 and 0.40 mT, are just the same as  $a_N$  and  $a_{3H}$  of DMPOX. In PDMPO, the chiral atom, C4, being coupling with one hydrogen atom and one phenyl group, will give a different hyperfine coupling constants for two  $\beta$ -protons, which should bring two values, 0.40 and 0.30 mT. It is, therefore, the resultant radical can be assigned as a DMPOX-type radical, 4-phenyl-5,5-dimethyl-1-pyrrolid-2-one-N-oxyl (PDMPOX).

As the reaction proceeded, red-brown colored fine particles were deposited on the inner surface of the vessel. Deposited particles could be dissolved only in  $\text{HNO}_3\text{-HCl}$  (1 : 3) solution (aqua regia), not in other mineral acids. The resultant solution contained  $\text{AuCl}_4^-$  species, which was recognized by the absorbance at 322 nm [24] and the inductively coupled plasma quantometry. Thus, the deposited particles was concluded to be those of Au(0). These results indicate that Au(III) in the reaction mixture was reduced to Au(0) through the 3-electron oxidation of the nitrones to DMPOX-type free radicals. The reaction between DMPO and  $\text{HAuCl}_4$  was conducted in  $\text{N}_2$ -bubbling water. No clear difference from the aspects of the reaction in ordinal water was observed. These results suggested that oxygen does not play an important role in the reaction.

In the reaction mixtures of  $\text{HAuCl}_4$  and non-cyclic nitrones, such as *N-tert-butyl- $\alpha$ -phenyl-nitrone* (PBN) and  *$\alpha$ -(4-pyridyl-1-oxide)-*N-tert-butyl-nitrone** (POBN), no radical species, such as *N-tert-butyl- $\alpha$ -phenyl-nitroxyl* (PBNX) and  *$\alpha$ -(4-pyridyl-1-oxide)-*N-tert-butyl nitroxyl** (POBNX), were observed. These results indicated that the reaction proceeds only with cyclic nitrones, such as DMPO, PDMPO, and M4PO.

No ESR signal was observed for both neutral and acidic solutions of DMPO in the presence of excess amounts of sodium dicyanoaurate(I). The redox potentials are  $E\{\text{Au(III)-Au(0)}\} = +1.0$ ,  $E\{\text{Au(III)-Au(I)}\} = +0.93$ ,  $E\{\text{Au(I)-Au(0)}\} = +1.15$  V for  $\text{Cl}^-$ ,  $E\{\text{Au(I)-Au(0)}\} = -0.61$  V for  $\text{CN}^-$ . These values indicated that  $\text{Au(III)Cl}_4^-$  is far easily reduced to Au(0) than  $\text{Au(CN)}_2^-$ . Furthermore,  $\text{CN}^-$  in the dicyanoaurate(I), being very stable

under neutral condition ( $\beta_2 = 11.23$ ) [26], cannot replace with DMPO. While the disproportionation of Au(I) ion is difficult to occur (Equation 2).



Because for  $\text{L} = \text{CN}^-$  the equilibrium constant of the equation 8 may be  $10^{-25}$ , and the disproportionation is not favorable in the present system [20]. These results suggest that the oxidation of DMPO to DMPOX coupled only with the three electrons reduction of Au(III) ion to Au(0).

### Aspects of DMPOX-type radical formation in cyclic nitrones-HAuCl<sub>4</sub> solutions

**Time course of radical formation:** The time course of DMPOX-type radical formation was examined using the mixture of nitrones (1.25 mM) and HAuCl<sub>4</sub> (1.25 mM) at pH 4. The ESR signal intensities of all DMPOX-type radicals examined increased with the elapse of time, and reached a maximum value within 20 ~ 30 min for DMPOX and PDMPOX, and more than 60 min for M4PO (Fig. 3). No other free radical species, except DMPOX-type ones, were observed over the period of 2 h after the reaction was started.

As shown in Fig. 3, the time courses of DMPOX-type radical formation in these nitrones show lag phases in the first 5 -10 min, which suggest complicated reaction processes. The decrease of Au(III) concentration in the reaction mixtures was fitted to equation (1) for the second order reaction kinetics (Fig. 3),

$$[\text{Au(III)}] = C/(C \cdot k \cdot t + 1) \quad (1)$$

where C is the initial Au(III) concentration (1.25 mM) and k, the equilibrium constant. The k values for each nitron are listed in Table 2. As shown in Table 2, the relative order of magnitude of rate for the initial stage of the reaction was PDMPO > DMPO >> M4PO, and that for the final radical formation, PDMPO  $\geq$  DMPO > M4PO. In PDMPO, a weak inductive effect proceeds the reaction, while in M4PO, a couple of methyl group at C3 strongly



hindered the reaction.

**Effect of nitron concentration:** Effect of nitron concentration on DMPOX-type radical formation was examined using the mixture of DMPO (0.5 ~ 5 mM) and H<sub>2</sub>AuCl<sub>4</sub> (1.25 mM) at pH 3. As shown in Fig. 4, a peculiar time dependency was observed in DMPOX radical formation. An anomalous increase in DMPOX was observed within first 10 min in the mixture of DMPO (2.5 ~ 5 mM) and H<sub>2</sub>AuCl<sub>4</sub> (1.25 mM). Twenty minutes after the reaction started, their time dependency settled down to those of low DMPO concentration (0.5 and 1.25 mM). These results suggested that excess amounts of DMPO eliminated the DMPOX radical formed in the mixture. Thoronally *et al.* reported the similar rapid decrease of DMPOX in the *tert*-butyl hydroperoxide/methaemoglobin systems [14], and suggested that DMPOX was decomposed to various products such as 5,5-dimethyl-1-hydroxy-2-pyrrolidone (DMPOXH), 4-methyl-4-nitrosopentanoic acid (MNPA), 4-methyl-4-nitropentanoic acid (MNOPA), 4-methyl-4-nitrosobutane, and a radical species, N-(5,5'-dimethyl-1'-oxyl-pyrrolidine)-4-hydroxylamino-4-methyl-pentane (DMPO-PNO) [25]. In the present system, an ESR signal of DMPO-PNO with 18 lines characterized by  $a_{N1} = 1.43$ ,  $a_H = 1.62$ ,  $a_{N2} = 0.42$  mT was not observed under excess DMPO condition. Furthermore, DMPOX decreased slower under small amounts of DMPO than that under excess DMPO condition (Fig. 3). Other possibilities are the reduction of DMPOX to its hydroxyl amine and the dimerization of DMPOX. Further precise experiments will be needed. In this paper, lower concentration, such as 1.25 mM of DMPO, where the anomalous increase was not observed with in first 10 min, will be adopted for following experiments.

**Effect of co-existing salts:** Addition of sodium chloride to the reaction mixture markedly decreased the ESR signal intensities of DMPOX, PDMPOX, and M4POX, while no effect

was observed by the addition of  $\text{NaNO}_3$  (Fig. 5). The amounts of  $\text{AuCl}_4^-$  in the solution, being depicted in Fig. 5, were calculated using the formation constants listed by Sillen and Martell [26]. As shown in Fig. 5, the formation of stable complex anion,  $\text{AuCl}_4^-$ , suppressed the red-ox reaction.

**Effect of solution pH:** The ESR signal intensities of DMPOX-type radicals decreased below pH 1 and above pH 6 (Fig. 6) in DMPO, PDMPO and M4PO. The ratios of chemical species,  $\text{AuCl}_4^-$ ,  $\text{Au}(\text{OH})\text{Cl}_3^-$ ,  $\text{Au}(\text{OH})_2\text{Cl}_2^-$ ,  $\text{Au}(\text{OH})_3\text{Cl}^-$  and  $\text{Au}(\text{OH})_4^-$ , calculated using the formation constants listed by Sillen and Martell [26], were also shown in Figs. 6. As shown in Fig. 6, as the ratios of  $\text{AuCl}_4^-$  and  $\text{Au}(\text{OH})_4^-$  in the reaction mixture were increased, the ESR signal intensities were decreased. Below pH 2, excess amounts of  $\text{Cl}^-$  stabilize the  $\text{AuCl}_4^-$  ion, while above pH 7, excess amounts of  $\text{OH}^-$  stabilize the  $\text{Au}(\text{OH})_4^-$  ion. In the intermediate pH range,  $\text{Cl}^-$  ion in the complex ions easily replace with  $\text{OH}^-$  ion. When DMPO exists in the solution,  $\text{Cl}^-$  ion in the complex ions easily replace with DMPO, in similar manner as the reaction between thiomorpholin-3-one and  $\text{AuCl}_4^-$  ion proposed by De Filippo et al. [27]. the complex formation between DMPO and Au(III) ion will proceed the redox reaction. These results suggested that the formation of stable complex anions, such as  $\text{AuCl}_4^-$  and  $\text{Au}(\text{OH})_4^-$ , suppressed the redox reaction.

### **Reaction of DMPOX's precursor with $\text{HAuCl}_4$**

1-Hydroxy-5,5-dimethyl-1-pyrrolid-2-one (HDMPN), the precursor of DMPOX in the oxidation of DMPO by  $\text{Fe}^{3+}$  ion [5] and in the electrochemical oxidation of DMPO [18,19], was also oxidized to DMPOX by Au(III) ion. The ESR signal intensity of DMPOX in the 1 : 1 mixture of HDMPN and  $\text{HAuCl}_4$  (1.25 mM) was increased with the time course (Fig. 7). The time course of Au(III) reduction in the system also described equation (1), where  $k =$

0.028 and the maximum amounts of free radical was 0.012 mM. The complexation between Au(III) and HDMPN is slower than that between Au(III) and DMPO. No precipitation of Au(0) was observed in the course of the reaction, which showed that the reduction step from Au(I) to Au(0) did not use for the HDMPN oxidation in the 1 : 1 mixture. On the other hand, in the 3 : 1 mixture of HDMPN (1.25 mM) and HAuCl<sub>4</sub> (0.417 mM), DMPOX was resulted with Au(0) precipitation, which indicated that the reduction step from Au(I) to Au(0) was coupled to the HDMPN oxidation. Summarizing these results, it is assumed that the three electrons-reduction process from Au(III) to Au(0) can be coupled with the oxidation of DMPO to DMPOX.

Weak ESR signal of DMPOX was observed in sodium dicyanoaurate(I)-HDMPN mixture solution under acidic condition (pH 0.5), despite no signal at neutral pH. There are two ways to explain the results. First is the oxidation ability of Au(I) ion. As dicyanoaurate(I) ion is very stable under neutral condition ( $\beta_2 = 11.23$ ) [26], HDMPN cannot form a complex with Au(I) against cyanide ion. However, under acidic condition, small amounts CN<sup>-</sup> in dicyanoaurate(I) ion replace with HDMPN, which causes the electron transfer from Au(I) to HDMPN and forms DMPOX. Second is the disproportionation of Au(I) ion (Equation 2 in the previous section) [20]. As discussed in the previous section, the disproportionation is not favourable for dicyanoaurate(I) ion [20]. These results, therefore, indicated the possibility that the reduction step from Au(I) to Au(0) was used to HDMPN oxidation.

## **Discussion**

Cyclic nitrones, such as DMPO, PDMPO and M4PO, were oxidized to DMPOX-type free radicals by Au(III) ion with the precipitation of Au(0). The reaction was heavily hindered by chloride ion, and was depressed in acidic and basic solutions. Assumed overall reaction

scheme for DMPO-HAuCl<sub>4</sub> system is shown in Fig. 8a. First, DMPO---Au(III)Cl<sub>3</sub> (complex I) is formed by the ligand exchange interaction between Cl<sup>-</sup> and DMPO, in a similar manner to the formation of the Fe<sup>3+</sup>-DMPO complex [5]. This is followed by the intra-molecular electron transfer from DMPO to Au(III)Cl<sub>3</sub> (complex II). The resultant complex II is converted to complex III (DMPO-OH---Au(II)Cl<sub>2</sub>) by the nucleophilic addition of water molecule at C2 atom. The resultant complex III is converted to complex IV (HDMPN---Au(I)Cl) by the intra-molecular electron transfer from DMPO-OH to Au(II)Cl<sub>2</sub> with HCl leaving. Finally, complex IV is converted to DMPOX by the intra-molecular electron transfer from HDMPN to Au(I)Cl and HCl and Au(0) release. Three electrons are, therefore, moved from DMPO to Au(III) through the reaction. When stable complex anions, such as AuCl<sub>4</sub><sup>-</sup> or Au(OH)<sub>4</sub><sup>-</sup>, are formed by the addition of HCl, NaCl or NaOH, the ligand exchange interaction in the first stage will be depressed. The rate of total Au(III) decrease corresponded to the increase of the ESR signal intensity of the resultant radical. Thus, the formation of complex I should be the rate determining step in the reaction process. Two methyl groups at C3 atom in M4PO sterically hinder the addition of water molecule to the C2 atom, which causes the slower reaction rate. Similar kinetics of M4PO oxidation were found in the Fe<sup>3+</sup>-M4PO system [27]. The weak inductive effect of 4-phenyl group in PDMPO will cause the slightly faster reaction rate than DMPO.

In the reaction mixture of HAuCl<sub>4</sub> and non-cyclic nitrones, such as PBN and POBN, no ESR signal was observed. Similar results were observed in the Fe<sup>3+</sup>-PBN system and in the electrochemical redox reaction of PBN [19]. These results suggested that the steric hindrance of the phenyl group in PBN or 4-pyridyl-1-oxide group in POBN inhibits the nucleophilic addition of water molecule and the chelation to the metal ion (Fig. 8b). Ozawa *et al.* reported the formation of PBNX and POBNX by the oxidation of PBN and POBN with ClO<sub>2</sub> radical [9]. They explained the reaction as the direct oxidation of α-C atom in PBN and POBN by

ClO<sub>2</sub> radical, which is quite different from our scheme.

As discussed above, the results reported herein can be well explained by the proposed reaction mechanism shown in Fig. 8a. The reduced form of DMPOX, HDMPN [18,19], was also oxidized to DMPOX by the reaction with 1/3 molar HAuCl<sub>4</sub>, indicating that every step of gold reduction, occurring from Au(III) to Au(0), is coupled with oxidation of HDMPN and DMPO. These results suggested the possibility of HDMPN being the DMPOX precursor in our system. In the case of the first step involved in HAuCl<sub>4</sub>-DMPO system, namely the ligand exchange interaction and the nucleophilic addition of water molecule, will proceed with the rate constant according to the time dependency of  $[1 - \exp(-0.043 \cdot t)]$ . Since the total Au(III) decrease should be caused by the ligand exchange interaction, the nucleophilic addition of water molecule, the electron transfer, and the complex III formation, the increase in DMPOX formation is little bit faster than the total Au(III) decrease,  $\exp(-0.070 \cdot t)$ . However, the complexation between Au(III) and HDMPN is slower than that between Au(III) and DMPO. The decrease of Au(III) in the DMPO-HAuCl<sub>4</sub> solution is faster than that in the HDMPN-HAuCl<sub>4</sub> solution, while the formation of DMPOX in the former solution is slower than that in the latter solution. The amounts of radical formed in the system (0.014 mM) (Table 2) is far smaller than the initial DMPO concentration (1.25 mM), which leads to the independency of Au(III) decrease and DMPOX formation. The reaction process from DMPO to HDMPN is little bit complicated, which causes the slower DMPOX formation in the DMPO-HAuCl<sub>4</sub> solution than that in the HDMPN-HAuCl<sub>4</sub> solution.

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## ABBREVIATIONS

ESR—electron spin resonance

DMPO—5,5-dimethyl-1-pyrroline-N-oxide

M4PO—3,3,5,5-tetramethyl-1-pyrroline-N-oxide

PBN—N-*tert*-butyl- $\alpha$ -phenyl-nitrone

POBN— $\alpha$ -(4-pyridyl-1-oxide)-N-*tert*-butyl-nitrone

PDMPO—4-phenyl-5,5-dimethyl-1-pyrroline-N-oxide

HDMPN—1-hydroxy-5,5-dimethyl-1-pyrrolid-2-one

DMPOX—5,5-dimethyl-1-pyrrolid-2-one-N-oxyl

PDMPOX—4-phenyl-5,5-dimethyl-1-pyrrolid-2-one-N-oxyl

M4POX—3,3,5,5-tetramethyl-1-pyrrolid-2-one-N-oxyl

PBNX—N-*tert*-butyl- $\alpha$ -phenyl-nitroxyl

POBNX— $\alpha$ -(4-pyridyl-1-oxide)-N-*tert*-butyl nitroxyl



Table 1. Hyperfine coupling constants of free radicals observed in the reaction mixtures of hydrogen tetrachloroaurate(III) and cyclic nitrones, DMPO, PDMPO and M4PO.

Free radicals	Hyperfine coupling constant A/g $\beta$ (mT)	
	$a_N$	$a_{3H}$ *
DMPOX	0.71	0.40 **
PDMPOX	0.71	0.40 ***
		0.30 ***
M4POX	0.67	

\* The hyperfine coupling constant for protons combined with C3 atom in nitron.

\*\* Two protons combined with C3 atom are equivalent.

\*\*\* Two protons combined with C3 atom are not equivalent each other.

Table 2. Kinetic parameters for DMPOX-type radical formation

Nitron	k	Maximum amounts of free radical (mM)
DMPO	0.043	0.014
PDMPO	0.069	0.015
M4PO	0.0061	0.010

$$[\text{Au(III)}] = C/(C \cdot k \cdot t + 1)$$

The initial concentration of each nitron was 1.25 mM.

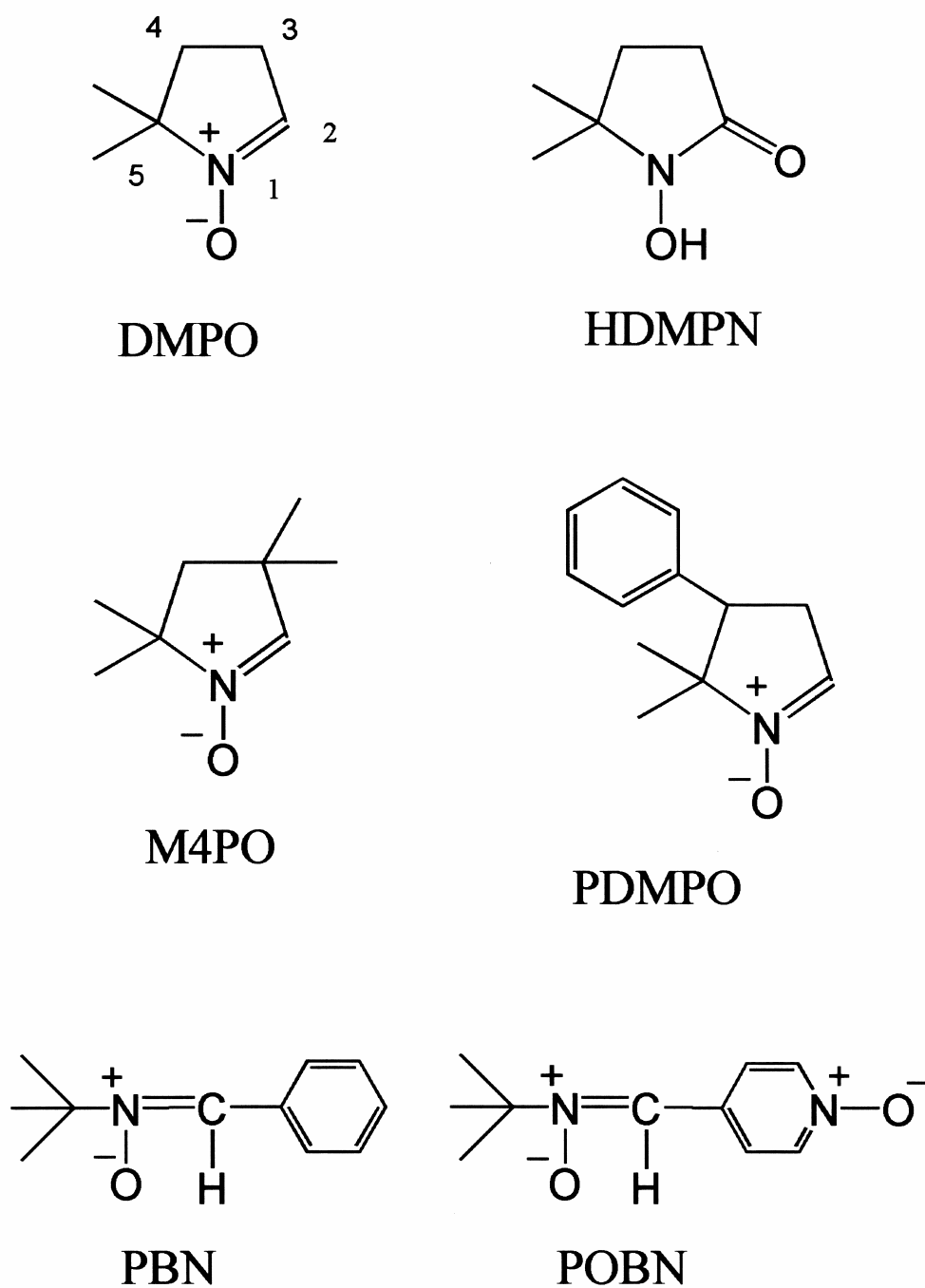


Fig. 1. Molecular structure of nitron-type spin traps and HDMPN, the precursor of DMPOX.

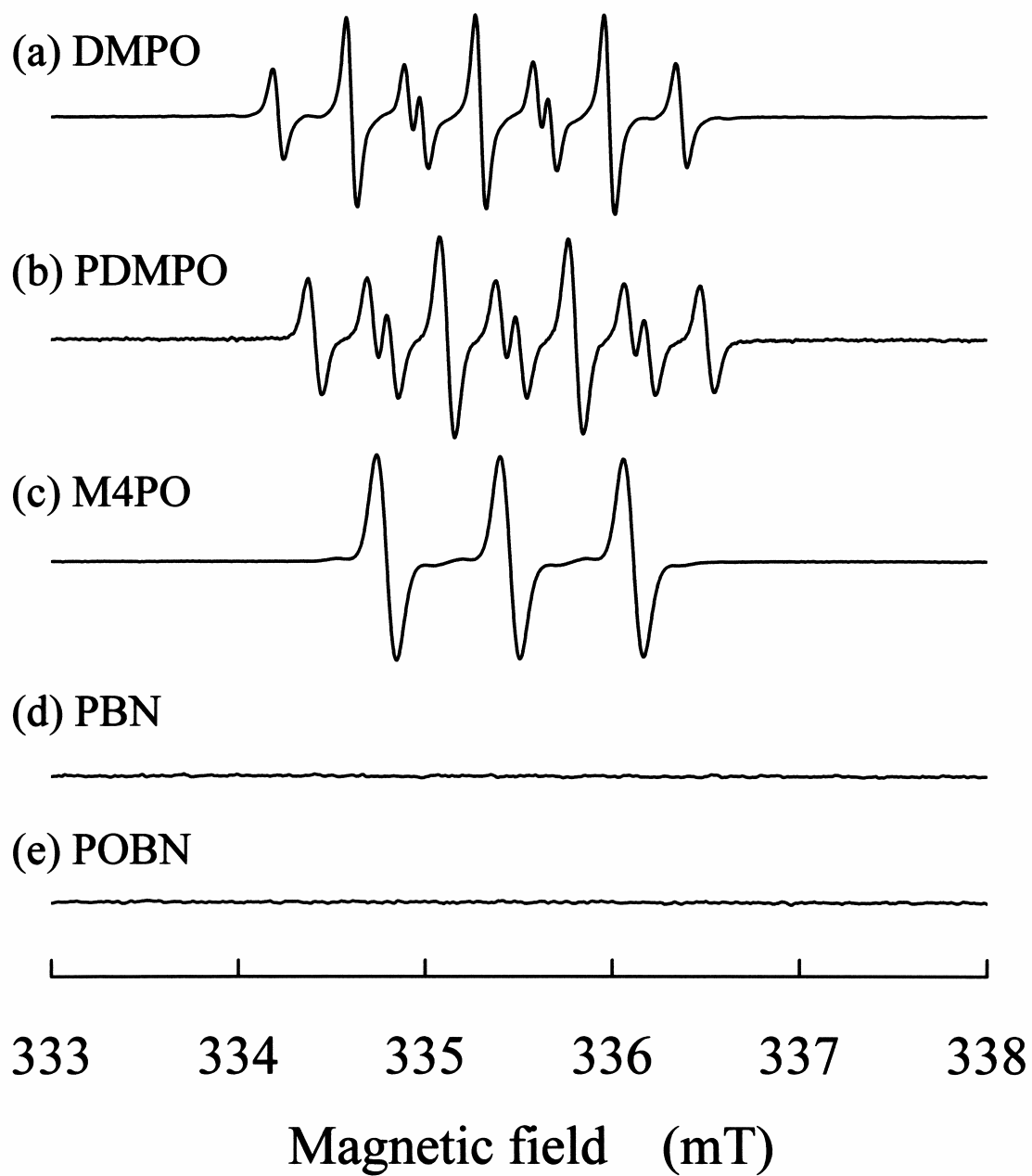


Fig. 2. ESR spectra of free radicals observed in the mixture solutions containing nitrones, DMPO (a), PDMPO (b), M4PO (c), PBN (d) and POBN (e) (1.25 mM) and  $\text{HAuCl}_4$  (1.25 mM). The microwave frequency was 9.42 GHz.

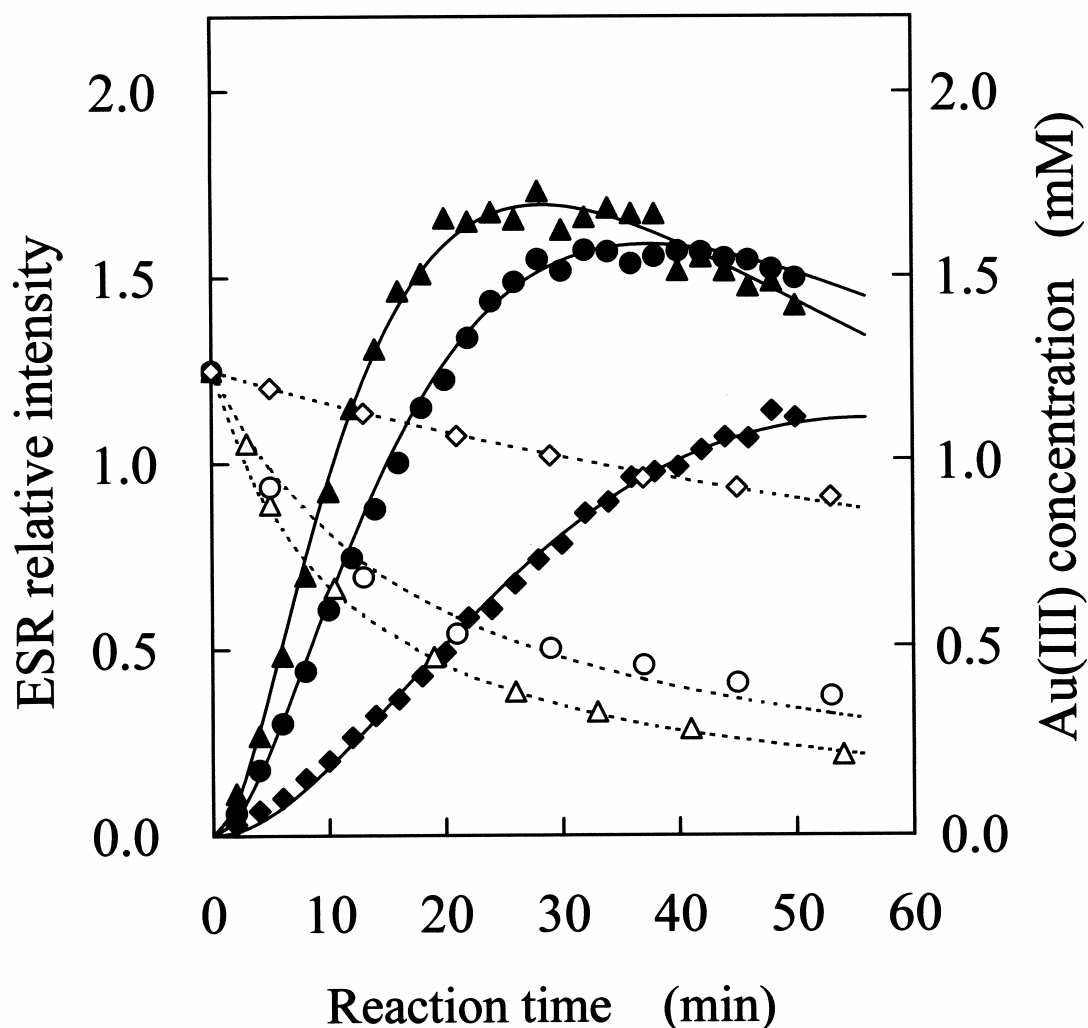


Fig. 3. Time course of ESR signal intensities and Au(III) concentration in the mixtures of nitrones (1.25 mM) and  $\text{HAuCl}_4$  (1.25 mM). Closed circle indicates the ESR signal intensity in DMPO, closed triangle, that in PDMPO, closed diamond, that in M4PO solutions, open circle, the Au(III) concentration in DMPO, open triangle, that in PDMPO, and open diamond, that in M4PO solutions. The solid lines indicated the best fitting curves using the least square method, and the dotted lines, those estimated using the equation,  $[\text{Au(III)}] = C/(C \cdot k \cdot t + 1)$ . Parallel experiments were conducted to determine gold concentrations as described in Experimental.

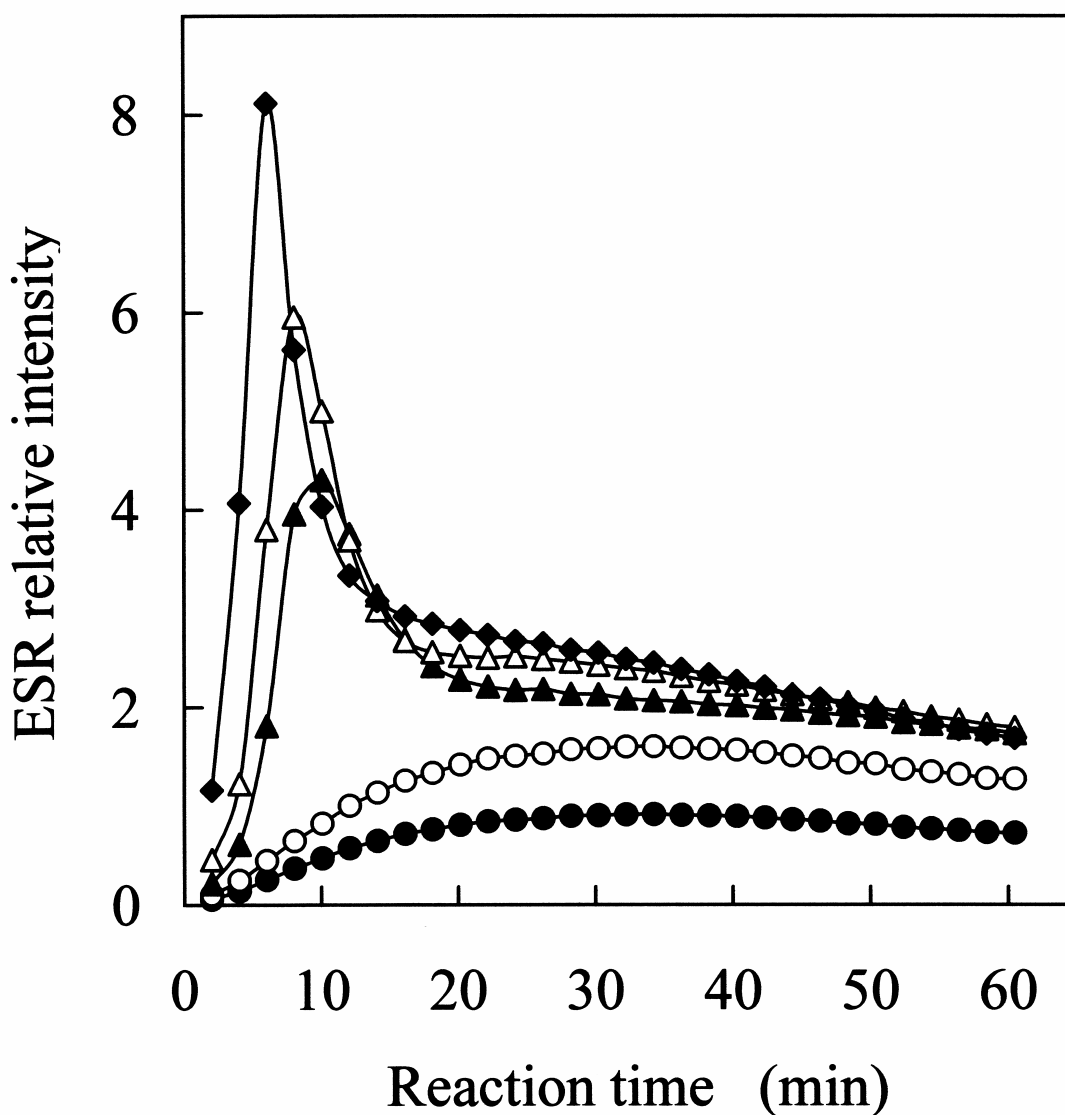


Fig. 4. Effect of DMPO concentration on the DMPOX formation in the mixtures of DMPO (0.5 ~ 5 mM) and  $\text{HAuCl}_4$  (1.25 mM). Closed circle indicate 0.5 mM, open circle, 1.25 mM, closed triangle, 2.5 mM, open triangle, 3.75 mM, and closed diamond, 5 mM DMPO solutions. The solid lines indicated the best fitting curves using the least square method.

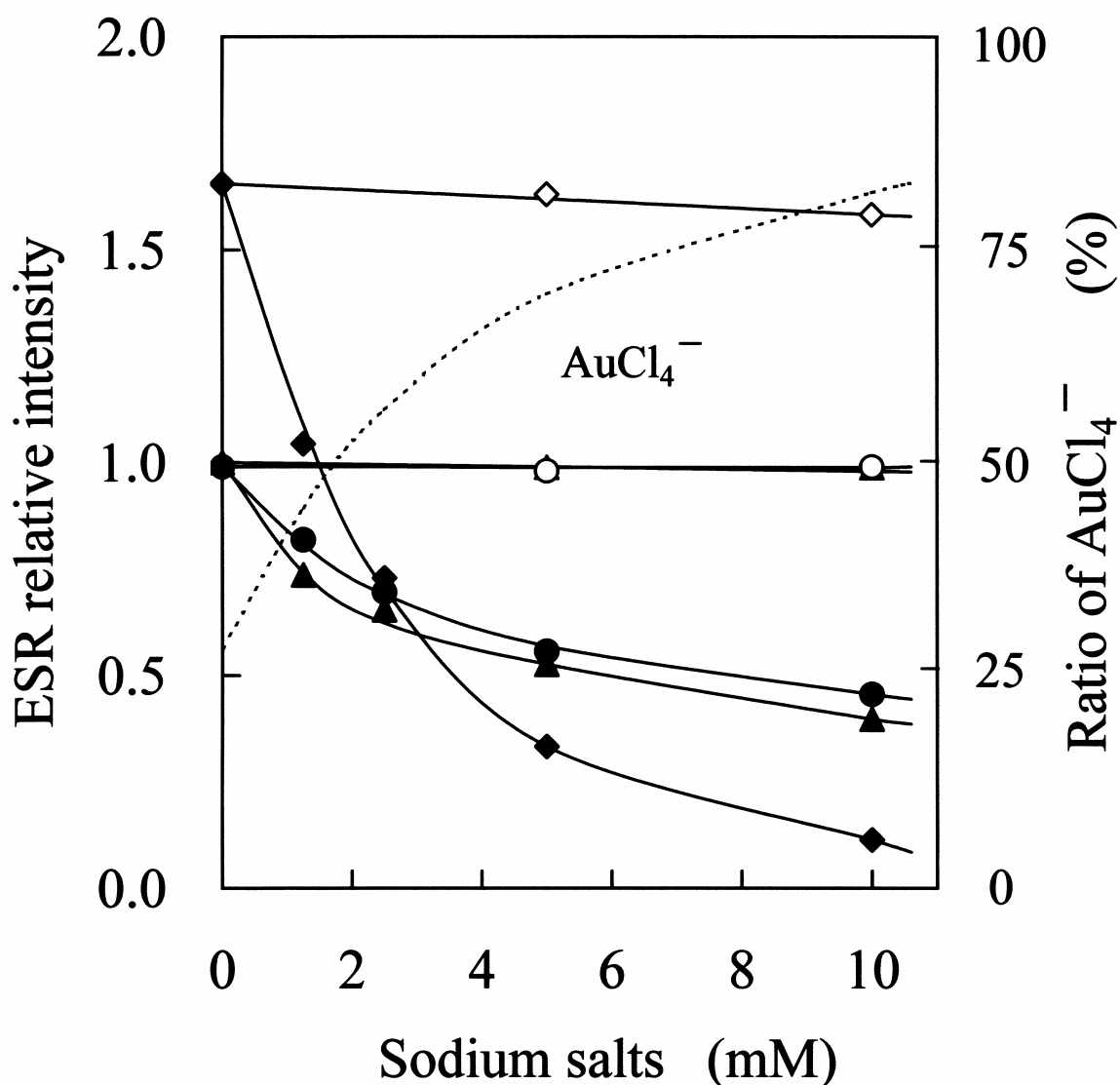


Fig. 5. Effect of sodium salts on ESR signal intensities in the mixtures of nitrones (1.25 mM),  $\text{HAuCl}_4$  (1.25 mM) and the desired amounts of sodium salts. The reaction time was 1h. Closed circle indicates the ESR signal intensity in DMPO-NaCl solution, open circle, DMPO- $\text{NaNO}_3$ , closed triangle, DMPO-NaCl, open triangle, PDMPO- $\text{NaNO}_3$  (superimposed on open circles), closed diamond, M4PO-NaCl and open diamond, M4PO- $\text{NaNO}_3$  solutions. Dotted line indicates the ratio of  $[\text{AuCl}_4^-]$  calculated using the formation constants listed by Sillen and Martell [26].

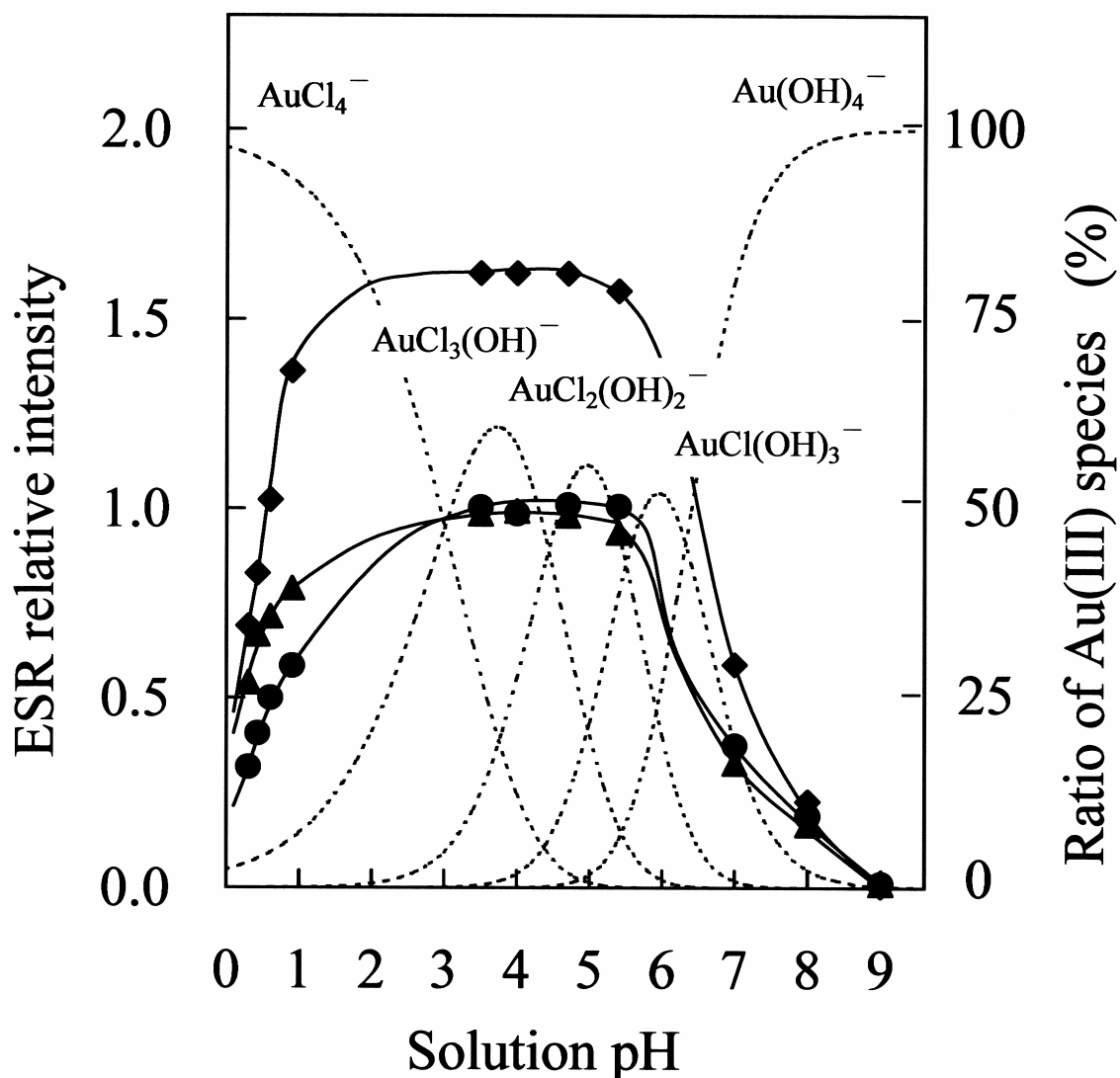


Fig. 6. Effect of solution pH on the ESR signal intensities in the mixtures of nitrones (1.25 mM) and  $\text{HAuCl}_4$  (1.25 mM). The reaction time was 1 h. Closed circle indicates the ESR signal intensity of DMPOX, closed triangle, that of PDMPOX, closed diamond, that of M4POX, and closed square, that of DEPMPOX. Dotted lines indicate the ratios of  $\text{AuCl}_4^-$ ,  $\text{AuCl}_3(\text{OH})^-$ ,  $\text{AuCl}_2(\text{OH})_2^-$ ,  $\text{AuCl}(\text{OH})_3^-$  and  $\text{Au}(\text{OH})_4^-$  calculated as described in the legend to Fig. 5.



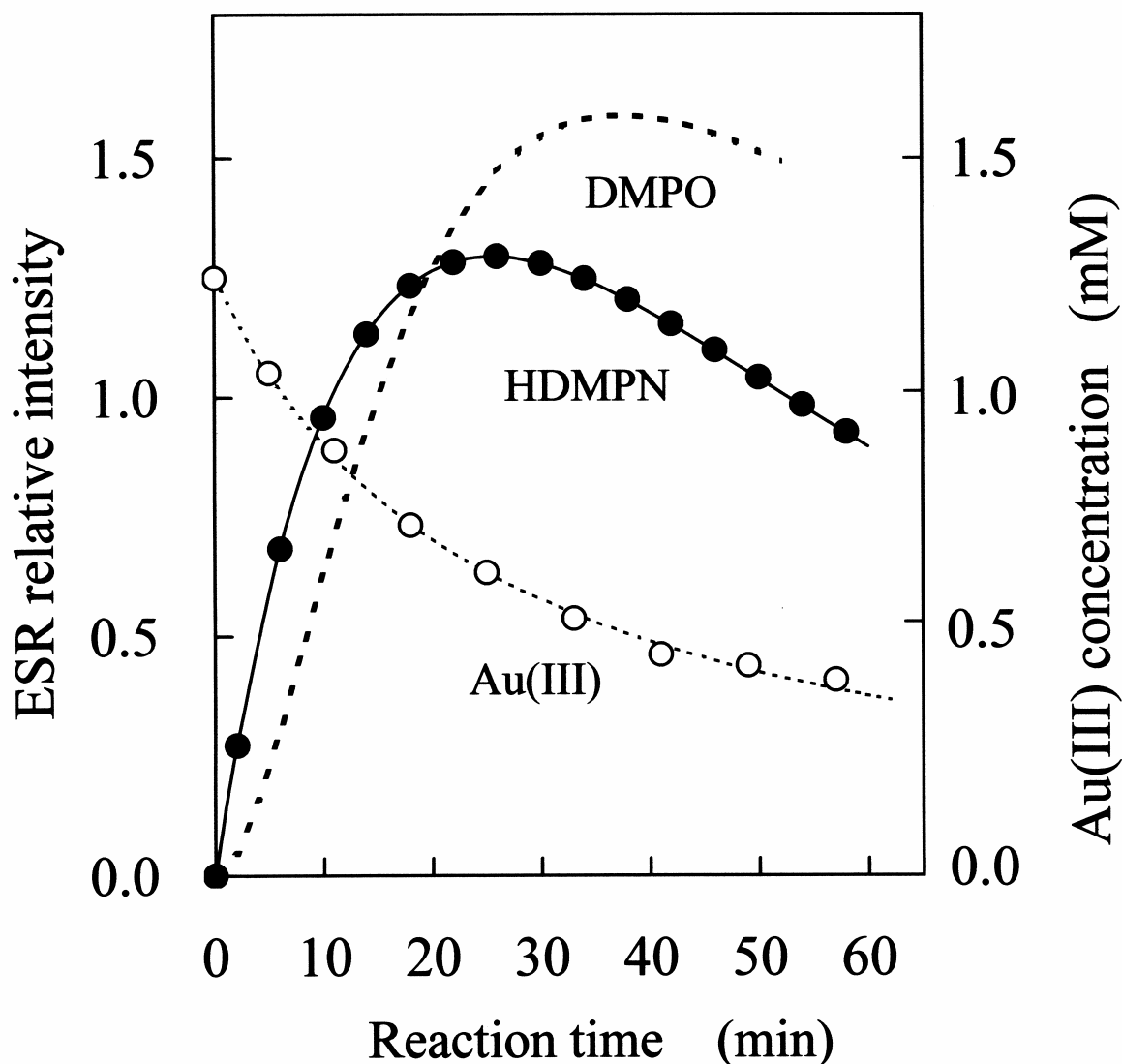
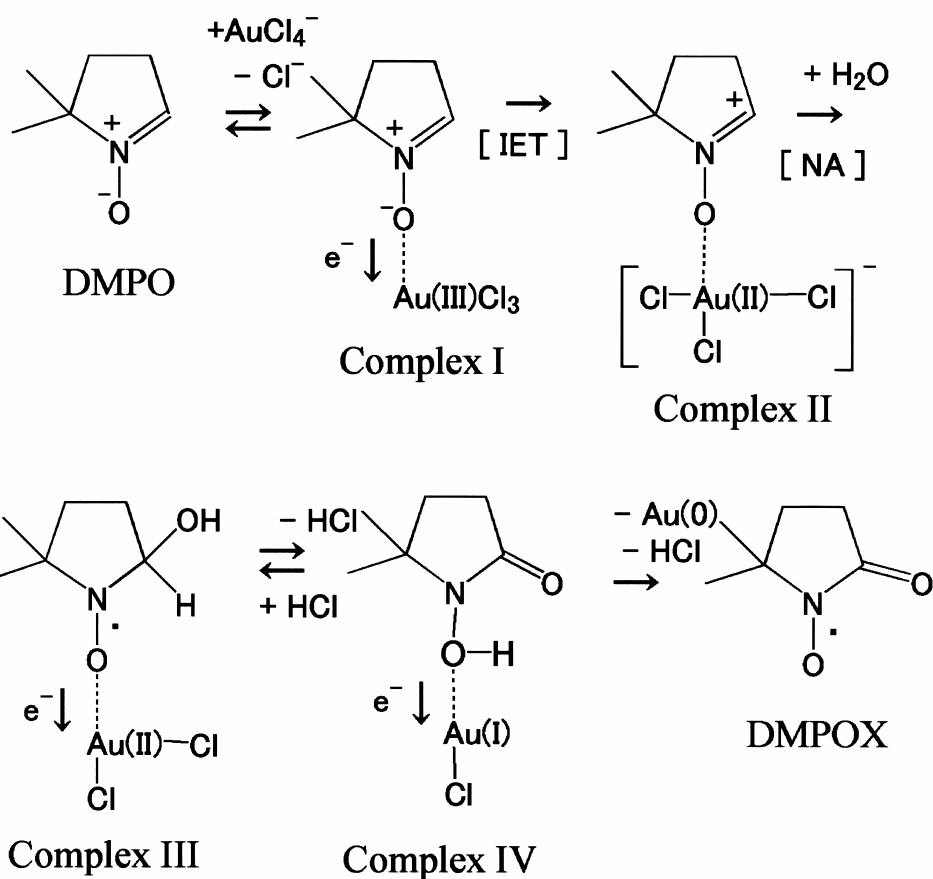


Fig. 7. Time course of ESR relative intensities of DMPOX observed in the mixture solution of  $\text{HAuCl}_4$  and HDMPN.  $\text{HAuCl}_4$  (1.25 mM) was mixed with DMPO and HDMPN (1.25 mM), respectively. Closed circle indicates the ESR intensity and open circle, the Au(III) concentration in the HDMPN- $\text{HAuCl}_4$  solution in the solution. The solid lines indicated the best fitting curve using the least square method, and the dotted lines, those estimated using the equation,  $[\text{Au(III)}] = C/(C \cdot k \cdot t + 1)$ . Dashed line indicates the ESR intensity in the DMPO- $\text{HAuCl}_4$  solution. Parallel experiments were conducted to determine gold concentrations as described in Experimental.

(a) Cyclic nitrones



(b) Non-cyclic nitronns

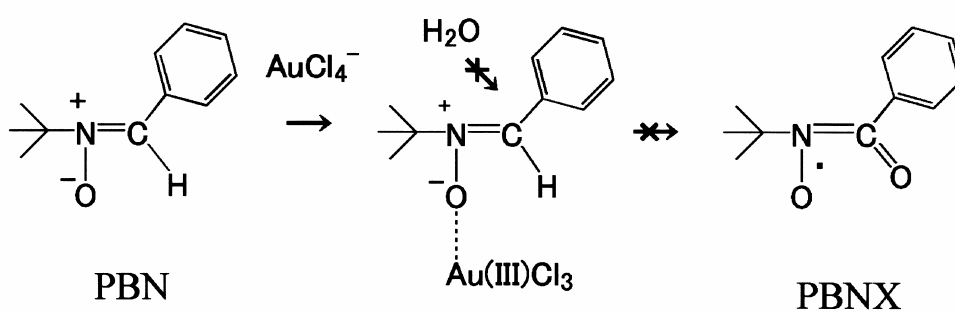


Fig. 8. Overall reaction of  $\text{HAuCl}_4$  with cyclic nitrones such as DMPO (a), and non-cyclic nitronns such as PBN (b). The intra-molecular electron transfer is abbreviated as IET, and the nucleophilic addition, as NA.

June 11, 2005

Dear Dr. Nakajima:

I am pleased to inform you that your manuscript entitled Electron spin resonance analysis of the oxidation reactions of nitron-type spin traps with gold(III) ion has been accepted for publication and will appear in a forthcoming issue of the Canadian Journal of Chemistry.

Please note: the Senior Editor's Office will require the Tables to be sent as a separate file from the manuscript. Please send via e-mail attachment to [jmurdoch@uwo.ca](mailto:jmurdoch@uwo.ca) as soon as possible.

As well, please complete the attached copyright agreement and fax to: 001-519-850-2371

Many thanks for your contribution to the journal.

Yours sincerely,  
Chris Orvig  
Editor