

## Enhancing Effect of Organic Substances on Hydroxyl Radical Generation During Ozonation of Water: Stopped-Flow ESR Technique

Sang-Kuk Han

Department of Marine Environmental Engineering, Faculty of Ocean System Engineering,  
Mokpo National Maritime University, Chukyo, Mokpo 530-729, Korea

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Generation of hydroxyl radical, one of their major active species in ozonation of water was directly observed with spin-trapping/electron spin resonance (ESR) technique using 5,5-dimethyl-pyrrolidine-1-oxyl (DMPO) as a spin-trapping reagent. Hydroxyl radical was trapped with DMPO as a stable radical, DMPO-OH. 80 mM of ozone produced  $1.08 \times 10^{-6}$  M of DMPO-OH, indicating that 1.4% of  $\cdot\text{OH}$  is trapped with DMPO if  $\cdot\text{OH}$  is produced stoichiometrically from ozone. Humic acid suppressed DMPO-OH generation in a dose-dependent manner. Generation rate of DMPO-OH was determined with ESR/stopped-flow measurement. Phenol derivatives increased the amount and generation rate of DMPO-OH, indicating that phenol derivatives enhance  $\cdot\text{OH}$  generation during ozonation of water.

**Key Words :** Ozonation, Phenol derivatives, Spin trapping ESR, Stopped flow, Hydroxyl radical

### Introduction

Ozonation is widely employed to remove musty smells in tap water purification. Various active oxygen species and free radicals are generated during ozonation and the most reactive specie of ozonation is reported to be hydroxyl radical.<sup>1</sup> The efficiency of ozonation depends on the kind and amount of dissolved substance in water, since some of them consume  $\cdot\text{OH}$  directly, suppress or enhance  $\cdot\text{OH}$  generation during ozonation of water.<sup>2</sup> On the other hand, ozonation produces various toxic substances from dissolved organic ones during water treatment processes. Thus, it is important for ozonation in water purification process to determine quantitatively the interaction among ozone, hydroxyl radical, and dissolved substances during ozonation.

Spin-trapping/electron spin resonance (ESR) technique has been developed to detect unstable radicals,<sup>3</sup> and revealed directly the generation of  $\cdot\text{OH}$  in Fenton reaction. We have previously observed the generation of  $\cdot\text{OH}$  during ozonation of water by spin trapping/ESR technique.<sup>4</sup> In this paper, we applied spin trapping/ESR measurement to determination of effects of humic acid and phenols on  $\cdot\text{OH}$  generation during ozonation. We also obtained kinetics data of  $\cdot\text{OH}$  generation using ESR/stopped-flow measurement, and discussed the enhancing effect of phenol derivatives on  $\cdot\text{OH}$  generation.

### Experimental Section

**Chemicals.** 2-Chloro or 2-methylphenol and humic acid were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). The initial concentration of humic acid was prepared by using the appropriate amount of humic acid sodium salts supplied by Kanto Chemical company. The solution of

humic acid mixed overnight. 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO) was purchased from Labotech Co. Ltd. (Tokyo, Japan) and stored at  $-20$  °C. Other chemicals were of the highest grade commercially available. Ozone consuming substances in distilled water was removed by pre-ozonation and the treated water was left until aqueous ozone was completely disappeared. All chemicals were dissolved in the water just before use.

**Aqueous ozone.** Ozone gas was produced from highly pure oxygen (Fukuoka Oxygen Co. Ltd. Fukuoka, Japan) with an ozone generator (PO-10, Fuji Electric Co. Ltd. Kanagawa, Japan). Aqueous solution containing ozone was prepared by continuous bubbling of distilled water with ozone gas through a absorber glass at 20 °C.

Concentration of aqueous ozone was determined by Indigo method.<sup>5</sup>

**ESR measurements.** The experimental procedure of spin trapping/ESR technique was described in our previous paper.<sup>4</sup> Phenol derivatives (10, 20, 40  $\mu\text{M}$ ) or humic acid solution (0.01 and 0.1 mg/mL) were mixed with DMPO (100 mM) in phosphate buffer (0.1 M, pH 7.4). Immediately after addition of aqueous ozone, 15  $\mu\text{L}$  of the sample solution were transferred into a capillary tube, and then the ESR spectrum was measured at room temperature with an ESR spectrometer (RE-1X, JEOL, Tokyo, Japan) at 10 mW of microwave (9.438 GHz) and 0.063 mT of field modulation (100 kHz). Hyperfine splitting constant of ESR spectrum was calibrated with  $\text{Mn}^{2+}$ , and the radical concentration was determined by comparing the signal intensity with that of a standard solution of diphenyl-2-picrylhydrazyl (DPPH).

ESR/stopped-flow system (Ohtsuka Electric Co. Ltd. Osaka, Japan) was used to determine the initial velocity of DMPO-OH generation. The solution containing DMPO (100 mM) and phenol derivatives (10, 20, 40  $\mu\text{M}$ ) in 0.1 M of phosphate buffer was quickly mixed with aqueous ozone using stopped-flow system, and then the spectra were

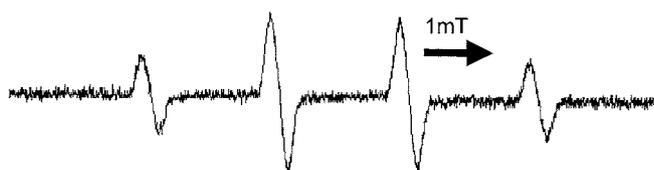
\*Tel: +82-61-240-7236; Fax: +82-61-240-7284; e-mail: skhan@mmu.ac.kr

obtained at the interval of 2 or 5 sec with an ESR spectrometer. A stopped-flow system was designed which allows precise control of flow variables during chemical generation of free radical species in the cavity of an ESR spectrometer. It may be used to determine the kinetics of disappearance of short-lived intermediates.

### Results and Discussion

Figure 1 shows the typical ESR spectrum 5 min after mixing aqueous ozone with DMPO. The spectrum was composed of quartet lines having peak height ratio of 1 : 2 : 2 : 1, and the parameters were hyperfine constant  $a_N = a_H = 1.50$  mT and  $g$ -value = 2.0055. These parameters coincided with those of DMPO-OH adduct as demonstrated previously.<sup>6</sup> The signal intensity was decreased by  $\cdot\text{OH}$  scavengers such as mannitol and thiourea (data not shown). The facts confirm the quartal signals to be DMPO-OH adduct.

The concentration of DMPO-OH adduct was determined by comparing the signal intensity with that of a standard. The amount of DMPO-OH increased gradually, and reached plateau almost 60 min after mixing. The linear relation was observed between the amount of DMPO-OH at plateau and the ozone concentration (Table 1), and 80  $\mu\text{M}$  of ozone produced 1.08  $\mu\text{M}$  of DMPO-OH. This indicates that 1.4% of  $\cdot\text{OH}$  generated for 60 min was trapped with DMPO, if  $\cdot\text{OH}$  is stoichiometrically produced from ozone. The  $\cdot\text{OH}$  yield (1.4%) obtained in this study is a great difference with the earlier measurements of 35% from  $^{60}\text{Co}$  radiolysis<sup>7</sup> and 33% from hydrogen peroxide photolysis studies.<sup>8</sup> But, it unclear why the  $\cdot\text{OH}$  trap yield in ozonation is different from that of radiolysis or photolysis.

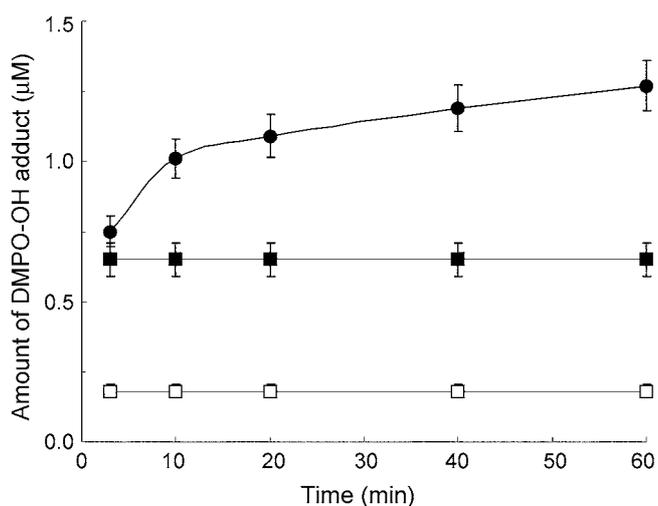


**Figure 1.** Typical ESR spectrum DMPO adduct during ozonation of water containing DMPO as a spin-trapper. Aqueous ozone (100  $\mu\text{M}$ ), and then the ESR spectrum was measured at room temperature with an ESR spectrometer (RE-1X, JEOL, Tokyo, Japan) as described in method.

The addition of humic acid decreased the amount of DMPO-OH in a dose-dependent manner (Figure 2). The consuming effect of humic acid on  $\cdot\text{OH}$  was estimated to be about  $10^{-5}$  mol  $\cdot\text{OH}/\text{mg}$  humic acid.

ESR/stopped-flow experiment was carried out to determine the generation rate of DMPO-OH adduct. Aqueous ozone was quickly introduced into DMPO solution containing phenol derivatives using stopped-flow system, and then ESR spectra were obtained at 2 or 5 sec after mixing.

Figure 3 shows the time-course of DMPO-OH generation after ozonation of the solution containing DMPO and 2-methylphenol. The amount of DMPO-OH increased gradually and reached plateau in all cases. The addition of 2-methylphenol increased not only the amount of DMPO-OH at plateau but also the rate of DMPO-OH generation. In this experiment, the concentration of DMPO (100 mM) is 2500 times higher than that of ozone (40  $\mu\text{M}$ ). The semilogarithmic plots of  $\{[\text{DMPO-OH}]_{\infty} - [\text{DMPO-OH}]_t\} / [\text{DMPO-OH}]_{\infty}$  versus time gave a linear line in all cases, where  $[\text{DMPO-OH}]_{\infty}$  is the final amount of DMPO-OH at plateau and  $[\text{DMPO-OH}]_t$  is the amount of DMPO-OH at  $t$  sec. The reaction of  $^2\text{OH}$  with DMPO is reported to be second-order kinetic and its

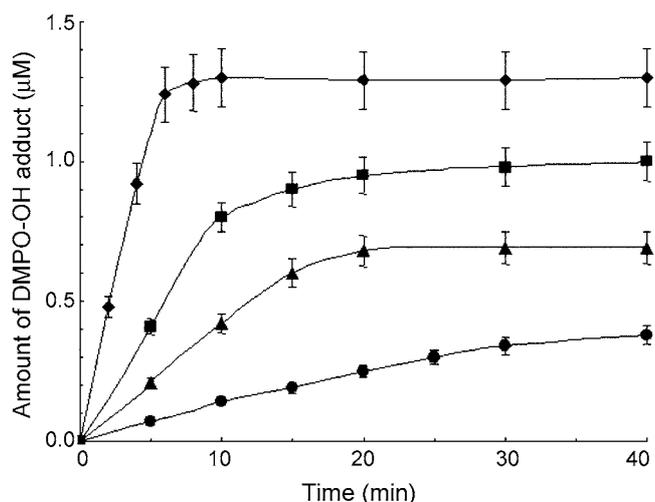


**Figure 2.** Effect of humic acid on DMPO-OH generation during ozonation of water. Aqueous ozone (100  $\mu\text{M}$ ) was added to the solution of DMPO (100 mM) and humic acid (0 (●), 0.01 (■), and 0.1 mg/mL (□)) at room temperature, and then examined with an ESR spectrometer as described in the legend of Figure 1. The data were mean  $\pm$  SD (n=3).

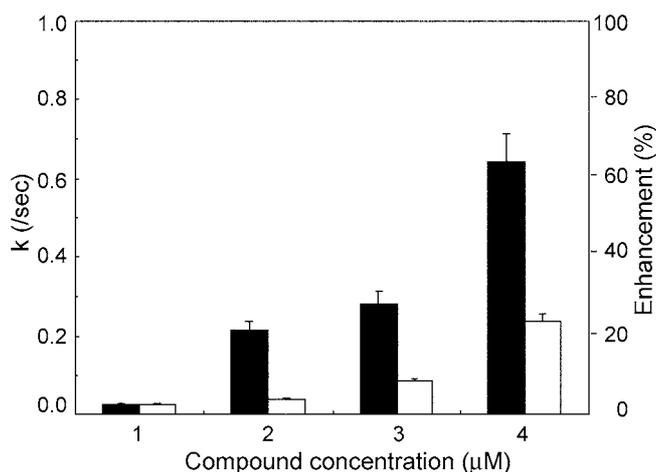
**Table 1.** Amounts of DMPO-OH adduct generated during ozonation of water

| Ozone conc. ( $\mu\text{M}$ )<br>Time (min) | 20                                  | 40              | 60              | 80              | 100             |
|---|-------------------------------------|-----------------|-----------------|-----------------|-----------------|
|   | Amount of DMPO-OH ( $\mu\text{M}$ ) |                 |                 |                 |                 |
| 10  | 0.33 $\pm$ 0.10                     | 0.54 $\pm$ 0.05 | 0.68 $\pm$ 0.03 | 0.81 $\pm$ 0.06 | 1.01 $\pm$ 0.11 |
| 20  | 0.36 $\pm$ 0.06                     | 0.58 $\pm$ 0.03 | 0.71 $\pm$ 0.07 | 0.88 $\pm$ 0.06 | 1.09 $\pm$ 0.17 |
| 40  | 0.39 $\pm$ 0.10                     | 0.63 $\pm$ 0.02 | 0.78 $\pm$ 0.04 | 1.01 $\pm$ 0.07 | 1.19 $\pm$ 0.11 |
| 60  | 0.41 $\pm$ 0.04                     | 0.63 $\pm$ 0.08 | 0.81 $\pm$ 0.11 | 1.08 $\pm$ 0.08 | 1.27 $\pm$ 0.14 |
| 70  | 0.41 $\pm$ 0.05                     | 0.62 $\pm$ 0.10 | 0.81 $\pm$ 0.09 | 1.09 $\pm$ 0.12 | 1.28 $\pm$ 0.14 |

Aqueous ozone was added to water containing 100 mM DMPO at room temperature, and then examined with an ESR spectrometer as described in the legend of Figure 1. The amounts of DMPO-OH adduct were determined from the signal intensity as described in method. The results were mean  $\pm$  SD (n = 4).



**Figure 3.** Effect of 2-methylphenol on the generation of DMPO-OH adduct during ozonation of water. The solution of DMPO (100 mM) and 2-methylphenol (0, 10, 20, 40  $\mu\text{M}$ ) using a stopped-flow system at room temperature, and then ESR spectrum was observed at  $\pm 0.75$  mT of sweep width and 2 or 5 sec of sweep time. The results were mean  $\pm$  SD ( $n = 4$ ).



**Figure 4.** Enhancing effect of phenol derivatives on the generation rate of DMPO-OH adduct during ozonation. The generation rates of DMPO-OH in the presence of 2-methyl ( $\square$ ) and 2-chlorophenol ( $\blacksquare$ ) were obtained as described in the legend of Figure 3. The results were mean  $\pm$  SD ( $n = 4$ ).

rate is  $2.1 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ ,<sup>9</sup> which is much faster than that of DMPO-OH generation shown in Figure 3.  $[\text{DMPO-OH}]_{\infty}$  may be interpreted as “total amount of  $\cdot\text{OH}$  capable to be trapped with DMPO” during ozonation, and  $[\text{DMPO-OH}]_t$  should be closely related to the total amount of  $\cdot\text{OH}$  generated till  $t$  sec. The decomposition of  $[\text{DMPO-OH}]_{\infty}$  that is, the generation of DMPO-OH obeyed first order kinetic, and the rate constants were calculated to be 0.026/s and 0.237/s for DMPO/aqueous ozone in the absence and presence of 40  $\mu\text{M}$  2-methylphenol, respectively. The results indicate that the addition of 2-methylphenol (40  $\mu\text{M}$ ) enhances the generation rate of DMPO-OH to ca 20-fold higher than without 2-methylphenol. Morioka *et al.*<sup>10</sup> reported that the direct reaction between phenols and ozone occurs readily

**Table 2.** The semilogarithmic plot (k/sec) for the generation of DMPO-OH during ozonation of water containing phenols

| Concentration ( $10^{-6}$ M) | Semilogarithmic plot (k/sec) |       |       |       |
|------------------------------|------------------------------|-------|-------|-------|
|                              | 0                            | 10    | 20    | 40    |
| Without phenols              | 0.026                        | –     | –     | –     |
| 2-chlorophenol               | –                            | 0.213 | 0.282 | 0.642 |
| 3-chlorophenol               | –                            | 0.197 | 0.269 | 0.496 |
| 4-chlorophenol               | –                            | 0.205 | 0.265 | 0.492 |
| 2,4-dichlorophenol           | –                            | 0.220 | 0.315 | 0.711 |
| 2,4,6-trichlorophenol        | –                            | 0.148 | 0.183 | 0.201 |
| 2-methylphenol               | –                            | 0.039 | 0.084 | 0.237 |
| 2,4-dimethylphenol           | –                            | 0.027 | 0.048 | 0.133 |
| 2,4,6-trimethylphenol        | –                            | 0.024 | 0.029 | 0.037 |

The numbers in the column of semilogarithmic plot indicate the decomposition rates obtained from the semilogarithmic plot of  $\{[\text{DMPO-OH}]_{\infty} - [\text{DMPO-OH}]_t\} / [\text{DMPO-OH}]_{\infty}$  against reaction time, where  $[\text{DMPO-OH}]_{\infty}$  is the amount of DMPO-OH at plateau and  $[\text{DMPO-OH}]_t$  is that at  $t$  sec. The results were mean  $\pm$  SD ( $n = 4$ ).

and we found that a semiquinone radical is formed from the reaction of phenol with ozone.<sup>11</sup> The formation of a semiquinone radical should enhance the superoxide anion radical.<sup>12</sup> These facts indicate the possibility that phenol changes the reaction mechanism of ozone in water. Also, the enhancing effect of DMPO-OH adduct in the presence of phenol agreed with an enhancing effect of organic substances by Staehelin and Hoigne,<sup>13</sup> or Ahn *et al.*<sup>14</sup>

Addition of phenol derivatives increased both the generation rate of DMPO-OH and the amount of DMPO-OH at plateau (data not shown). Figure 4 shows the generation rate of DMPO-OH in ozonation of water containing DMPO and phenol derivatives. The addition of 2-chlorophenol (40  $\mu\text{M}$ ) enhanced the rate to 0.64/s, which is ca 60-fold higher than that without phenol. These results suggest that both 2-methyl and 2-chlorophenol enhance  $\cdot\text{OH}$  generation in ozonated water and that the enhancing effect of 2-chlorophenol is much larger than that of 2-methylphenol.

Enhancing effect on DMPO-OH by phenol derivatives are summarized in Table 2. The enhancing effect of phenols on  $\cdot\text{OH}$  generation from aqueous ozone depended on the kind, position and number of substituents of phenol. The chlorine substitution of phenol had more enhancing effect on  $\cdot\text{OH}$  generation in aqueous ozone than that of the methyl substitution, and the ortho-substitution by chlorine showed stronger enhancement. 2,4-Dichlorophenol showed the strongest enhancing effect on  $\cdot\text{OH}$  generation among phenols examined, followed by 2-chlorophenol.

## Conclusions

Generation of  $\cdot\text{OH}$  in ozonation of water was determined as DMPO-OH generation with spin trapping/ESR/stopped-flow system. The presence of humic acid suppressed DMPO-OH generation, and  $\cdot\text{OH}$  consumed with humic acid is estimated to be about  $10^{-5}$  mol  $\cdot\text{OH}/\text{mg}$  humic acid. The addition of phenol derivatives enhanced both the rate and

final amount of DMPO-OH generation, and 2-chlorophenol (40  $\mu$ M) increased the rate to 60 times more than that without phenol. Also, the chlorophenol compounds had more enhancing effect on  $\cdot$ OH generation in aqueous ozone than that of the methylphenols.

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