Quantification of hydroxyl radical generation with spin trapping ESR technique in batch system

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Abstract Effect of phenols on hydroxyl radical formation during ozonation was investigated, using spin trap ESR technique in batch system. Addition of methylphenols increased amounts of DMPO-OH. The enhancement effect was similar to the case in which the stopped flow equipment was used. In case of chlorophenols, amount of DMPO-OH was also enhanced but it decreased in a time and ozone-concentration dependent manner. In case of 20 μM ozone, DMPO-OH generation in batch and stopped flow system were almost the same, when the enhancing effect was calculated using the Y intercept obtained by extrapolating from elimination curves. However, in higher ozone concentration, enhancing effects of phenols in DMPO-OH formation were underestimated compared with that in stopped flow-system. In conclusion, batch system, in addition with stopped-flow system, would be also useful for analyzing enhancing effects of dissolved compounds on ozone reaction when they have moderate enhancing effects.

Keywords Ozonation; hydroxyl radical; electron spin resonance; spin trapping

Introduction

Ozonation is widely employed to remove odors in tap water. Staehelin and Hoigne (1982), and Buhler et al., (1984) have revealed that ozonation is initiated by decomposition of O_3 with OH^- and propagated by the reaction of $\cdot\text{O}_2^-$ with O_3 to produce hydroxyl radical, $\cdot\text{OH}$. The complicated reaction cascade is proposed as SBH model (Staehelin et al., 1984). Hydroxyl radical is one of the most reactive species in ozonation of water (Hoigne, 1988). The generation or consumption $\cdot\text{OH}$ is influenced by the kind and amount of dissolved substances in water (Staehelin and Hoigne, 1985). Production of $\cdot\text{OH}$ have been estimated by measuring the reaction-products between $\cdot\text{OH}$ and $\cdot\text{OH}$ -probe, such as salicylate or 4-hydroxybenzoate. These methods are, however, poorly specific to $\cdot\text{OH}$ and inevitably suffer from artefacts by $\cdot\text{OH}$ independent production of the reaction-products. The generation of $\cdot\text{OH}$ have not been determined selectively or quantitatively, due to lack of the method.

Electron spin resonance (ESR) spectroscopy is a sensitive and selective method to detect free radicals, and spin-trapping/electron spin resonance (ESR) technique has been developed to detect unstable radicals (Janzen and Blackburn, 1969). We have directly determined the generation of $\cdot\text{OH}$ in water during ozonation by ESR technique using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin trapping reagent (Utsumi et al., 1994; Han et al., 1998a, 1998b). In our previous papers, we demonstrated that addition of phenols enhanced $\cdot\text{OH}$ generation during ozonation in stopped-flow system.

Measurement of $\cdot$ OH generation in batch style would be simple and easy method compared with that in stopped-flow style. It was, however, unknown that batch system could be applied to fast reaction of $\cdot$ OH generation especially for measuring the effect of dissolved organic compounds such as phenols.

In this study, generation of $\cdot$ OH were measured both in batch system and in stopped-flow system. Applicable experimental conditions of batch system for $\cdot$ OH measurement was investigated.

Methods

Chemicals

Phenol derivatives were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). 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 were removed by pre-ozonation, and the treated water was left till aqueous ozone was completely disappeared. All chemicals were dissolved in phosphate buffer (0.1 M, pH 7.4) of the water just before use. 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 ozone solution 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 (Bader and Hoigne, 1981).

ESR measurement

The experimental procedure of spin trapping/ESR technique was described in our previous paper (Utsumi et al., 1994). Phenol derivatives were dissolved with DMPO (100 mM) in phosphate buffer (0.1 M, pH 7.4). Immediately after addition of aqueous ozone, 15 μl 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 (100kHz). The hyperfine splitting constant and g-value were calculated with those of Mn^{2+} , and the radical concentration was determined by comparing the signal intensity with that of a standard solution of diphenyl-2-picrylhydrazyl (DPPH). Stopped-flow system (Ohtsuka Electric Co. Ltd., Osaka, Japan) was also used to determine the initial velocity of DMPO-OH generation. The solution containing DMPO (100 mM) and phenol derivatives (0, 10, 20, 40 μM) in 0.1 M of phosphate buffer was rapidly with aqueous using stopped-flow system, and then the spectra were obtained at the interval of 2 or 5 sec with X-band ESR.

Results and discussion

After mixing aqueous ozone with DMPO solution, ESR spectrum of quartet lines was observed. The ESR parameters coincided with those of DMPO-OH adduct as demonstrated previously (Utsumi et al., 1994). The amount of DMPO-OH increased gradually and reached plateau almost 60 min after mixing (Fig. 1). The linear relation was obtained between the amount of DMPO-OH at plateau and the ozone concentration.

This indicates that about 1.1 % of $\cdot\text{OH}$ generated for 60 min was trapped with DMPO, if one molecule of ozone produces one molecule of $\cdot\text{OH}$. The ratio obtained does not mean stoichiometric but semiquantitative generation of $\cdot\text{OH}$ contains complicated reactions and enhanced by chain reaction.

Fig.1. Time course of DMPO-OH formation during ozonation of water. Aqueous ozone concentration were, Δ 20 μM , $\square$ 60 μM and $\circ$ 100 μM .

Fig.2. Enhancement of DMPO-OH formation during ozonation (100 μM), in the absence ($\circ$) or presence of 0.04 mM 2,4,6-trimethylphenol ($\square$), 0.04 mM 2,4,6-trichlorophenol ($\bullet$).

DMPO-OH formations in the presence of phenols were also measured. In case of 2,4,6-trimethylphenol, amount of DMPO-OH was constant during the observation, which was quite different from that in the absence of phenols (Fig. 2). The amount of DMPO-OH increased depending on phenol concentration. Enhancement effects for $\cdot\text{OH}$

were 1.3 – 2.0 for 2,4-dimethylphenol and 1.2 – 1.5 for 2,4,6-trimethylphenol. These enhancements did not depend on ozone concentration (Table 1).

In stopped-flow system, however, enhancement effects for $\cdot\text{OH}$ were 1.4 – 2.9 for 2,4-dimethylphenol, 1.3 – 1.6 for 2,4,6-trimethylphenol and the enhancements depended on ozone concentration (Table 1). Observed enhancing effects for DMPO-OH were lower in batch system than that in stopped-flow system for same ozone concentrations. Relative enhancing effects for 2,4-dimethylphenol and 2,4,6-trimethylphenol were nearly the same in both systems.

Enhancing effects of chlorophenols, which we reported large DMPO-OH enhancing effects previously on stopped-flow system, were measured in batch and stopped-flow systems. At first observation, amount of DMPO-OH increased significantly in the presence of 2-chlorophenol (Fig. 2). However, amount of DMPO-OH decreased depending on reaction time. The decrements were remarkable for high ozone concentration. Since the decrement of DMPO-OH depended on ozone concentration, active species in ozonation or reaction intermediates of chlorophenol during ozonation would contribute for the decrement. In fact, we previously reported that DMPO-OH was converted to non-radical compounds by excess oxidation (Utsumi et al., 1994). So, $\cdot\text{OH}$, p-semiquinone radical generated during ozonation or superoxide radical generated from quinone cycle may contribute the decrement. Though we did not observe superoxide adduct of DMPO.

Table 1. Enhancement of DMPO-OH formation by methylphenols or chlorophenols during ozonation in batch and stopped-flow system. Enhancement ratio was calculated by dividing amount of DMPO-OH at plateau in the presence of phenol (40 μM), with that without phenols.

Ozone (μM)	20	40	60	100
<i>Batch system</i>				
Methyl phenol				
2,4-	1.3	2.0	1.5	1.8
2,4,6	1.5	1.2	1.2	1.4
Chlorophenol				
2,4-	4.4	4.4		
2,4,6-	2.1	2.7		
<i>Stopped-flow system</i>				
Methyl phenol				
2,4-	1.9	2.3		
2,4,6-	1.6	1.4		
Chlorophenol				
2,4-	5.0	11.1		
2,4,6-	2.7	3.3		

We then examined that enhancement effects for DMPO-OH could be evaluated using batch system, in case of large enhancing effects. For that purpose, the amount of

DMPO-OH at time zero was extrapolated from time course of DMPO-OH. The ratio of DMPO-OH in the presence of phenols was calculated and expressed as enhancing ratio in batch system (Table 1). On the other hand, enhancing effects for DMPO-OH in stopped-flow system was calculated by using enhancing ratio for initial velocity of DMPO-OH formation.

For lower ozone concentration, enhancing ratio of DMPO-OH by chlorophenols were nearly the same in both systems. However, higher enhancing ratios were observed in stopped-flow system than in batch system for higher ozone concentration. For example, addition of 40 μM of 2-chlorophenol enhanced DMPO-OH 9 times in stopped-flow system, but only 4.5 times in batch system. It was indicated that DMPO-OH decreased significantly in higher ozone concentration and that enhancing effects could not be evaluated properly in these conditions. Current results indicated that a simple and handy method of batch system can be applied for measuring $\cdot\text{OH}$ during ozonation when enhancing ratios by phenols were 5 times compared with that without phenols (Fig. 3).

Fig. 3. Relation between enhanced DMPO-OH formation during ozonation in the presence phenols, measured with stopped flow or batch system.

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