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# Hydroxyl radical generation by dissociation of water molecules during 1.65 MHz frequency ultrasound irradiation under aerobic conditions

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#### ABSTRACT

The dissociation of water molecules by ultrasound irradiation under aerobic conditions was demonstrated experimentally. To be able to detect the dissociation of water molecules, we performed the ultrasound irradiation of <sup>17</sup>O-labelled water (H<sub>2</sub><sup>17</sup>O) under aerobic conditions. The hydroxyl and hydrogen radicals generated during the ultrasound irradiation process were trapped with 5,5-dimethyl-1pyrroline-N-oxide (DMPO), and electron spin resonance (ESR) spectroscopy was performed on the DMPO spin adducts. In the ESR spectrum, a 15-line signal attributable to the trapping of the hydroxyl radicals containing <sup>17</sup>O (<sup>17</sup>OH radicals) by DMPO together with a 4-line signal attributable to the trapping of the hydroxyl radicals containing <sup>16</sup>O (<sup>16</sup>OH radicals) by DMPO were observed. The generation of <sup>17</sup>OH radicals indicated that H<sub>2</sub><sup>17</sup>O was dissociated by the sonolysis process under aerobic conditions. On the other hand, the ESR signal attributable to the trapping of hydrogen radicals by DMPO was not observed, suggesting that hydrogen radicals were not generated during the dissociation of water molecules.

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## 1. Introduction

Ultrasound is being used in medical and dental fields. Especially, ultrasound with the frequency above 1 MHz is used for therapeutic and cleaning tools [1-4]. In the therapeutic applications, the high frequency has been chosen due to its depth of penetration into tissue [5–7]. For ultrasound cleaning, the high frequency ultrasound is effective to remove particles from the surface of object such as tooth [8,9], which is due to a strong acceleration of the water molecules. However, there are concerns whether ultrasound irradiation can induce oxidative damage in the human body, because free radicals are generated when water is exposed to ultrasound waves with an intensity higher than a certain threshold. These free radicals have the ability to damage biological molecules (e.g., enzymes, DNA, and lipids) in vitro. Thus, to prevent any accidental damage resulting from the use of ultrasound waves and to ensure the safety and reliability of ultrasound irradiation with respect to its application in the medical and dental fields, a

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http://dx.doi.org/10.1016/j.bbrc.2016.12.171 0006-291X/© 2016 Published by Elsevier Inc. thorough understanding of the mechanism of free-radical generation under ultrasound irradiation is essential.

When bulk water is subjected to ultrasound irradiation, cavitation bubbles grow and collapse repeatedly [10]. During their collapse, the local temperature and pressure can be higher than 5000 K and 500 atm, respectively [11,12]. The high temperature and pressure are what result in the generation of free radicals [13–16]. The primary reaction responsible for the generation of free radicals is the thermal dissociation of the water molecule into a hydroxyl radical (•OH) and a hydrogen radical (•H), as shown below:

$$H_2 O \rightarrow \bullet OH + \bullet H \tag{1}$$

The reaction (1) has been confirmed in bulk water exposed by ultrasound with 50 kH frequency under argon gas conditions through electron spin resonance (ESR)-spin trapping measurements; the ESR signals attributable to hydroxyl radicals and hydrogen atoms were detected [15–17]. However, it is confirmed only under limited measurement conditions.

Under aerobic conditions, only the signal attributable to hydroxyl radicals has been observed [16,18]. Therefore, the reaction (1) may not be the primary reaction of water sonolysis under aerobic conditions. Alternatively, under aerobic conditions, oxygen

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molecules also undergo thermal dissociation in the interior of collapsing bubbles, resulting in biradical oxygen atoms (•O•) [19,20]:

$$O_2 \to 2 \bullet O \bullet \tag{2}$$

Based on the reactions involving oxygen atoms known from combustion chemistry, the following reactions have been proposed to explain the generation of hydroxyl radicals:

$$\bullet O \bullet + H_2 O \to \bullet O H + \bullet O H \tag{3}$$

In this reaction, only hydroxyl radicals are generated under ultrasound irradiation of water in the presence of oxygen molecules. Based on this reaction mechanism, the isotopic exchange of <sup>18</sup>Olabelled oxygen molecules (<sup>18</sup>O<sub>2</sub>) and water (H<sup>2</sup><sub>2</sub><sup>6</sup>O), and the generation of hydrogen peroxide labelled with one <sup>18</sup>O atom (H<sup>2</sup><sub>2</sub><sup>18</sup>O<sup>16</sup>O) under ultrasound irradiation can be explained [21]. Therefore, Reaction (2) and (3) are commonly accepted as the mechanism of hydroxyl radical generation by ultrasound irradiation of liquid water in the presence of air. However, to confirm that the proposed mechanism is correct, the primary reaction involved in the sonolysis of water under aerobic conditions, represented by Reaction (3), should be validated experimentally.

In this study, in order to elucidate the primary reaction involved in the sonolysis of water under aerobic conditions, we performed the ultrasound irradiation of <sup>17</sup>O-labelled air-containing water. The free radicals generated were detected using 5,5-dimethyl-1pyrroline-N-oxide (DMPO). The nuclei spin number of <sup>17</sup>O is 5/2, while that of <sup>16</sup>O is 0. Because of this difference in the spin numbers, the ESR signal attributable to <sup>17</sup>OH-trapping DMPO (DMPO-<sup>17</sup>OH) was different from that related to <sup>16</sup>OH-trapping DMPO (DMPO-<sup>16</sup>OH). This allowed the hydroxyl radicals generated from water and those from oxygen molecules to be distinguished. Further, ultrasound at 1.65 MHz frequency, which is used in therapeutic and cleaning tools in medical and dental fields [1–4], was used in the present study.

## 2. Materials and methods

DMPO was obtained from LABOTEC Co., Ltd. (Tokyo, Japan) while 2,2,6,6-teteramethyl piperidinol (TEMPOL) was obtained from Sigma-Aldrich (Tokyo, Japan).  $H_2^{17}O$  (>90 atom%  $^{17}O$ ) was purchased from NUKEM Isotopes GmbH (Alzenau, Germany).

A previously reported experimental device for ultrasound generation was used in this study [22,23]. In this device, the ultrasound generator is placed at the bottom of a water bath. First, 200  $\mu$ l of water containing 100 mM DMPO was transferred to a glass tube (15 mm in diameter and 85 mm in length). The glass tube was placed in the water bath and was fixed above the ultrasound generator. The temperature of the bulk water was controlled at 20  $\pm$  1 °C by the low temperature circulator (Tokyo Rikakikai Co., Ltd, Japan). Next, ultrasound irradiation (power of 30 W and frequency of 1.65 MHz) was performed for 30 s.

The water sample exposed to the ultrasound waves was transferred to a flat quartz cell ( $150 \ \mu$ L) for the ESR measurements. The optical path length was 0.25 mm. The ESR spectra were recorded at room temperature using an X-band spectrometer (JES-FA-100, JEOL Ltd., Tokyo, Japan), which was operated at 9.43 GHz. The magnetic field was modulated at 100 kHz. The conditions for measuring the ESR spectra were as follows: microwave power of 4 mW; magnetic field of 335.5  $\pm$  5.0 mT; field modulation width of 0.1 mT; sweep time of 2 min; and time constant of 0.1 s.

The signal intensities were normalized with respect to a MnO marker, and the concentrations of the stable radical products were

determined on the basis of the signal height using an external standard, TEMPOL. The concentration of OH was determined using Digital Data Processing (JEOL) and expressed in terms of the concentration of DMPO-OH, a spin adduct of OH. All the tests were performed at least in triplicate at room temperature.

## 3. Results and discussion

During the ultrasound irradiation to  $H_2O$  in the presence of air, a 4-line ESR signal with the intensity ratio 1:2:2:1 was observed, as shown in Fig. 1. This signal was attributable to DMPO-<sup>16</sup>OH, which has a g value of 2.0064 and hyperfine constants of 1.49 ( $a_N$ ) and 1.49 mT ( $a_H$ ) [24]. No signal related to hydrogen radicals was observed, in keeping with previous reports.<sup>16,18</sup> This result indicated that hydroxyl radicals were generated during the ultrasound irradiation to water under aerobic conditions in the present study, in agreement with previous studies.

The dependence of the generation of DMPO-OH on the ultrasound irradiation time is shown in Fig. 2. In keeping with what has been reported previously, the DMPO-OH signal increased linearly until an irradiation time of 30 s. For further increases, it plateaued, owing to the degradation of DMPO-OH because of the ultrasound waves [25]. To investigate the initial stage of the water sonolysis process (i.e., the stage prior to the degradation of DMPO-OH), we performed ultrasound irradiation for 30 s. In this case too, the ultrasound irradiation to H<sub>2</sub><sup>17</sup>O was performed under aerobic conditions. In addition to the 4-line signal attributable to DMPO-<sup>16</sup>OH, another multiple-lines signal was observed, as shown in Fig. 3(a). Using a simulation of the ESR measurement results, the signals shown in Fig. 3(a) could be fitted well using a combination of the 4line signal attributable to DMPO-<sup>16</sup>OH and a 15-line signal attributable to DMPO-<sup>17</sup>OH, as shown in Fig. 3(b). The 4-line signal related to DMPO-<sup>16</sup>OH has a g-value of 2.0064 and hyperfine constants of 1.49 mT  $(a_N)$  and 1.49 mT  $(a_H)$  (Fig. 3(c)), while the 15-line signal related to DMPO-<sup>17</sup>OH has a g-value of 2.0064 and hyperfine constants of 1.49 (a<sub>N</sub>), 1.47 mT (a<sub>H</sub>), and 0.47 mT (a<sub>170</sub>) (Fig. 3(d)) [24]. This result indicates that not only <sup>16</sup>OH radicals but also <sup>17</sup>OH radicals were generated by based on the results of the ESR measurements, we concluded that hydroxyl radicals are generated by the dissociation of water molecules when they are exposed to ultrasound waves under aerobic conditions.

According to the simulation results shown in Fig. 3(c) and (d), the ratio of the intensities of the DMPO-<sup>16</sup>OH and DMPO-<sup>17</sup>OH signals was nearly 1. This ratio was almost constant until the ultrasonic irradiation time was at least 45 s. These results indicate that the amounts of <sup>16</sup>OH and <sup>17</sup>OH radicals generated during water sonolysis are almost equal when ultrasound is exposed to H<sub>2</sub><sup>17</sup>O in



Fig. 1. ESR spectrum of the spin adduct formed during the sonolysis of  $H_2O$  for 30 s under aerobic conditions in the presence of 100 mM DMPO.

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Fig. 2. Effect of the sonication time on the yield of DMPO-OH under aerobic conditions.

the presence of  ${}^{16}O_2$ . Based on the proposed reaction mechanism for hydroxyl radical generation by water sonolysis under aerobic conditions [14,21], hydroxyl radical generation from  $H_2^{17}O$  by sonolysis in the presence of  ${}^{16}O_2$  can be described as follows:

$${}^{16}\text{O}_2 \to {}^{\bullet 16}\text{O}{}^{\bullet} + {}^{\bullet 16}\text{O}{}^{\bullet} \tag{4}$$



**Fig. 3.** ESR spectra of the spin adducts formed during the sonolysis of  $H_2^{17}$ O for 30 s under aerobic conditions in the presence of 100 mM DMPO. (a) Experimental spectrum, (b) simulated spectra of both DMPO-<sup>17</sup>OH and DMPO-<sup>16</sup>OH, (c) simulated spectrum of DMPO-<sup>17</sup>OH, and (d) simulated spectrum of DMPO-<sup>16</sup>OH.

$$\cdot^{16}O_{\bullet} + H_2^{17}O \to \cdot^{16}OH + \cdot^{17}OH$$
 (5)

3

According to this mechanism and the purity of  $H_2^{17}O$  used in this study, the ratio of the numbers of  $\cdot^{17}OH$  and  $\cdot^{16}OH$  generated by water sonolysis should be 1.0–1.2, a value that would be consistent with the ratio determined experimentally.

We did not detect the DMPO-H-related signal attributable to the trapping of the hydrogen radicals by DMPO during the sonolysis of water under aerobic conditions. Under the conditions used in this study, had hydrogen radicals been generated, a signal attributable to DMPO-H should have been detected, in spite of the short lifetime of DMPO-H. The reaction rate constant of hydrogen radicals with respect to oxygen molecules at around 298 K is approximately  $10^{10}$  M<sup>-1</sup> s<sup>-1</sup> [26–29], while that with respect to DMPO at around 298 K is approximately  $10^9 \text{ M}^{-1}\text{s}^{-1}$  [30–32]. In this study, 100 mM of DMPO was dissolved in water; this amount is approximately  $5 \times 10^2$  times higher than the concentration of the oxygen dissolved in water (approximately 200 µM). Thus, the hydrogen radicals generated probably reacted with DMPO more quickly than they did with the oxygen molecules. Given the difference in the reaction rates, that fact that no signal attributable to DMPO-H was detected can be interpreted as the two meanings as follows.

- 1) Hydrogen radicals are not generated during the ultrasound irradiation of water under aerobic conditions, as suggested by Reactions (4) and (5).
- Hydrogen radicals are generated and are rapidly scavenged by the oxygen molecules present in the gas bubbles when water is



**Fig. 4.** ESR spectra of the spin adducts formed during the sonolysis of  $H_2^{17}O$  for 30 s in an argon atmosphere in the presence of 100 mM DMPO. (a) Experimental spectrum, (b) simulated spectra of DMPO-H, (c) simulated spectrum of DMPO-<sup>17</sup>OH, (d) simulated spectrum of DMPO-<sup>16</sup>OH, and (e) simulated spectrum of the DMPO-trapping carbon-centered radical.

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subjected to ultrasound irradiation under aerobic conditions [19,35].

If hydrogen radicals are generated by the thermal dissociation of water as the mechanism 2), then <sup>17</sup>OH radicals are also generated from water. Further, an equivalent amount of <sup>16</sup>OH radicals can be generated by the following reaction:

$$H_2^{1/0} \to \bullet^{1/0} OH + \bullet H \tag{6}$$

 $\bullet H + {}^{16,16}O_2 \rightarrow \bullet {}^{16}O \bullet + \bullet {}^{16}O H \tag{7}$ 

The oxygen biradical generated in Reaction (7) can react with a water molecule as shown in Reaction (3). Even in this case, the ratio of the number of  $^{17}$ OH radicals to that of the  $^{16}$ OH radicals would be around 1, as observed in the experimental results described above.

Alternatively, there was a possibility that hydrogen radicals cannot be effectively trapped by DMPO in  $H_2^{17}$ O. In the previous work, the ESR signal due to DMPO-H was not observed in the sonolysis of  $H_2^{17}O$  in the presence of argon gas [17], though the signal was observed in the sonolysis of  $H_2^{16}O$ . However, we detected the generation of hydrogen radicals during the sonolysis of argoncontaining H<sub>2</sub><sup>7</sup>O. As shown in Fig. 4, distinct ESR signals related to DMPO-H, DMPO-<sup>17</sup>OH, and DMPO-<sup>16</sup>OH, which are attributable to the generation of hydrogen, <sup>17</sup>OH, and <sup>16</sup>OH radicals, respectively, were observed. Further, a low-intensity signal related to DMPO-C, which is attributable to the generation of <sup>16</sup>OH radicals and carbon-centered radicals, was also observed. The generation of hydrogen, hydroxyl, and carbon-centered radicals was also observed during the sonolysis of  $H_2^{16}O$  (data not shown). This result indicated that the isotopic substitution of the oxygen atom in the water molecule does not affect the dissociation of water molecules under ultrasound irradiation as depicted by Reaction (1), and not affect the ability of DMPO for trapping hydrogen radicals. No detection of the ESR signal due to DMPO-H is probably due to the difference in the frequency of the ultrasound waves used or/and the purity of  $H_2^{17}$ O also probably affect the amount of hydrogen radicals generated during ultrasound irradiation.

According to Reaction (1), the hydrogen and hydroxyl radicals would be generated in equal amounts. The rate constant for the reaction of DMPO with hydrogen radicals is almost equal to that for the reaction of DMPO with hydroxyl radical; however, the life time of DMPO-H is shorter than that of DMPO-OH. Therefore, the intensity of the DMPO-H signal was expected to be equivalent to or smaller than that of the DMPO-<sup>17</sup>OH signal. However, the intensity of the DMPO-H signal was approximately three times higher than that of the DMPO-<sup>17</sup>OH signal, which was also observed during the sonolysis of  $H_2^{16}O$  [18]. When sonolysis is performed in the presence of argon gas, the local temperature can be as high as approximately 1700 K; this is higher that the temperature for the case when sonolysis is performed in the presence of oxygen and nitrogen gases [33]. From combustion chemistry, it is known that the hydroxyl radical dissociates into the hydrogen radical and the oxygen biradical at such high temperatures, as follows [34]:

$$\bullet OH \to \bullet O\bullet + \bullet H \tag{8}$$

This dissociation reaction is probably what causes the amount of hydrogen radicals to be higher than the amount of hydroxyl radicals.

## 4. Conclusions

In this study, we performed the sonolysis of <sup>17</sup>O-labelled aircontaining water and measured the ESR spectra of the spin adducts attributable to the hydroxyl and hydroxyl radicals, which are expected to be generated during the dissociation of the water molecule. Based on the fact that an ESR signal attributable to <sup>17</sup>OH radicals was observed, we concluded that hydroxyl radicals are generated by the dissociation of water molecules during ultrasound irradiation under aerobic conditions. The dissociation of the water molecule under anaerobic conditions had been reported previously, wherein hydroxyl and hydrogen radicals were detected. However, we did not detect an ESR signal attributable to hydrogen radicals under aerobic conditions. Thus, the hydroxyl radical is probably generated via a reaction of the water molecule with the oxygen biradical (Reaction (5)), as proposed previously, or/and because of the thermal dissociation of the water molecule (Reaction (6)).

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## **Transparency document**

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#### References

- K.G. Baker, V.J. Robertson, F.A. Duck, A review of therapeutic ultrasound: biophysical effects, Phys. Ther. 81 (2001) 1351–1358.
- [2] D. Miller, N. Smith, M. Bailey, G. Czarnota, K. Hynynen, I. Makin, American institute of ultrasound in medicine bioeffects committee, overview of therapeutic ultrasound applications and safety considerations, J. Ultrasound Med. 31 (2012) 623–634.
- [3] A.R. Biesbrock, L.M. Bayuk, D.S. Yates, M.V. Santana, R.D. Bartizek, The clinical effectiveness of a novel power toothbrush and its impact on oral health, J. Contemp. Dent. Pract. 15 (2002) 1–10.
- [4] W.B.D. Forfang, B.H. You, I.-H. Song, Ultrasonic dental therapy: trends and prospects, Eur. Int. J. Sci. Tech. 2 (2013) 1–6.
- [5] D.O. Draper, J.C. Castel, D. Castel, Rate of temperature increase in human muscle during 1 MHz and 3 MHz continuous ultrasound, J. Orthop. Sports Phys. Ther. 22 (1995) 142–150.
- [6] G.R. ter Haar, Basic physics of therapeutic ultrasound, Physiotherapy 64 (1978) 100–103.
- [7] Ř.J. Baker, G.W. Bell, The effect of therapeutic modalities on blood flow in the human calf, J. Orthop. Sports Phys. Ther. 13 (1991) 23–27.
- [8] K. Shinada, L. Hashizume, K. Teraoka, N. Kurosaki, Effect of ultrasonic toothbrush on *Streptococcus* mutans, Jpn. J. Conserv. Dent. 42 (1999) 410–417.
  [9] M.R. Costa, V.C. Silva, M.N. Miqui, T. Sakima, D.M.P. Spolidorio, J.A. Cirelli,
- [9] M.R. Costa, V.C. Silva, M.N. Miqui, T. Sakima, D.M.P. Spolidorio, J.A. Cirelli, Efficacy of ultrasonic, electric and manual toothbrushes in patients with fixed orthodontic appliances, Angle Orthod. 77 (2007) 361–366.
- [10] G. R.ter. Haar, S. Daniels, Evidence for ultrasonically induced cavitation in vivo, Phys. Med. Biol. 26 (1981) 1145–1149.
- [11] K.S. Suslick, Sonochemistry, Science 247 (1990) 1439–1445.
- [12] E.B. Flint, K.S. Suslick, The temperature of cavitation, Science 253 (1991) 1397–1399.
- [13] K.S. Suslick, The chemical effects of ultrasound, Sci. Am. 260 (1989) 80-86.
- [14] P. Riesz, T. Kondo, Free radical formation induced by ultrasound and its biological implications, Free Rad. Biol. Med. 13 (1992) 247–270.
- [15] K. Makino, M.M. Mossoba, P. Riesz, Chemical effects of ultrasound on aqueous solutions. Evidence for •OH and •H by spin trapping, J. Am. Chem. Soc. 104 (1982) 3537–3539.
- [16] K. Makino, M.M. Mossoba, P. Riesz, Chemical effects of ultrasound on aqueous solutions. Formation of hydroxyl radicals and hydrogen atoms, J. Phys. Chem. 87 (1983) 1369–1377.
- [17] T. Kondo, C.M. Krishna, P. Riesz, Effect of non-volatile scavengers of hydroxyl radicals on thymine radical formation induced by gamma-rays and ultrasound, Int. J. Radiat. Biol. 53 (1988) 891–899.
- [18] M. Kohno, T. Mokudai, T. Ozawa, Y. Niwano, Free radical formation from sonolysis of water in the presence of different gases, J. Clin. Biochem. Nutr. 49 (2011) 96–102.
- [19] E. Hart, A. Henglein, Free radical and free atom reactions in the sonolysis of aqueous iodide and formate solutions, J. Phys. Chem. 89 (1985) 4342–4347.
- [20] M.A. Beckett, I. Hua, Elucidation of the 1,4-dioxane decomposition pathway at discrete ultrasonic frequencies, Environ. Sci. Technol. 34 (2000) 3944–3953.
- [21] C.H. Fischer, E.J. Hart, A. Henglein, Ultrasonic irradiation of water in the presence of <sup>18, 18</sup>O<sub>2</sub>: isotope exchange and isotopic distribution of H<sub>2</sub>O<sub>2</sub>,

Please cite this article in press as: A. Miyaji, et al., Hydroxyl radical generation by dissociation of water molecules during 1.65 MHz frequency ultrasound irradiation under aerobic conditions, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2016.12.171

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J. Phys. Chem. 90 (1986) 1954–1956.

- [22] A. Iwasawa, K. Saito, T. Mokudai, M. Kohno, T. Ozawa, Y. Niwano, Fungicidal action of hydroxyl radicals generated by ultrasound in water, J. Clin. Biochem. Nutr. 45 (2009) 214–218.
- [23] Y. Matsumura, A. Iwasawa, T. Kobayashi, T. Kamachi, T. Ozawa, M. Kohno, Detection of high-frequency ultrasound-induced singlet oxygen by the ESR spin-trapping method, Chem. Lett. 42 (2013) 1291–1293.
- [24] G.R. Buettner, Spin trapping: ESR parameters of spin adducts, Free Radic. Biol. Med. 3 (1987) 259–303.
- [25] S.N. Nam, S.K. Han, J.W. Kang, H. Choi, Kinetics and mechanisms of the sonolytic destruction of non-volatile organic compounds: investigation of the sonochemical reaction zone using several OH\* monitoring techniques, Ultrason. Sonochem. 10 (2003) 139–147.
- [26] G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (•OH/•O<sup>-</sup>) in aqueous solution, J. Phys. Chem. Data Ref. 17 (1988) 513–886.
- [27] J. Elliot, D.R. McCracken, G.V. Buxton, N.D. Wood, Estimation of rate constants for near-diffusion-controlled reactions in water at high temperatures, J. Chem. Soc. Faraday Trans. 86 (1990) 1539–1547.

- [28] J. Elliot, A pulse radiolysis study of the temperature dependence of reactions involving H, OH and eaq. in aqueous solutions, Radiat. Phys. Chem. 34 (1989) 753-758.
- [29] S. Gordon, E.J. Hart, J.K. Thomas, The ultra violet spectra of transients produced in the radiolysis of aqueous solutions, J. Phys. Chem. 68 (1964) 1262–1264.
- [30] E. Finkelstein, G.M. Rosen, E.J. Rauckman, Spin trapping of superoxide and hydroxyl radical: practical aspects, Arch. Biochem. Biophys. 200 (1980) 1–16.
- [31] E. Finkelstein, G.M. Rosen, EJ. Rauckman, Spin trapping, Kinetics of the reaction of superoxide and hydroxyl radicals with nitrones, J. Am. Chem. Soc. 102 (1980) 4994–4999.
- [32] P.R. Marriott, M.J. Perkins, D. Griller, Spin trapping for hydroxyl in water: a kinetic evaluation of two popular traps, Can. J. Chem. 58 (1980) 803–807.
- [33] F.R. Young, Sonoluminescence from water containing dissolved gases, J. Acoust. Soc. Am. 60 (1976) 100–104.
- [34] J. Colussi, L.K. Weavers, M.R. Hoffmann, Chemical bubble dynamics and quantitative sonochemistry, J. Phys. Chem. A 102 (1998) 6927–6934.
  [35] M.H. Uddin, S. Hatanaka, S. Hayashi, Effects of aqueous temperature on
- [35] M.H. Uddin, S. Hatanaka, S. Hayashi, Effects of aqueous temperature on sonolysis of bisphenol A: rate constants increasing with temperature under oxygen, J. Chem. Eng. Jpn. 42 (2009) 303–308.

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