International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 07 (B); July 2019; Page No.19438-19442
DOI: http://dx.doi.org/10.24327/ijcar.2019.19442.3750

Research Article

NANOBUBBLE MIXED SOLUTION OF CARBON DIOXIDE AND HYDROGEN FOR REMOVING HYDROXYL AND SUPEROXIDE ANION FREE RADICALS IN WATER

Toyohisa Fujita[1]., Lanyin Zhang[2]., Josiane Ponou[2]., Gjergj Dodbiba[2]., Zhenyao Han[1]., Yuezhou Wei[1]*., Hiromi Kurokawa[3] and Hirofumi Matsui[3]

[1]College of Resources, Environment and Materials, Guangxi University, China
[2]Graduate School of Engineering, the University of Tokyo, Japan
[3]Doctoral Programs in Medical Sciences, the University of Tsukuba, Japan

ARTICLE INFO

Article History:

Received 12th April, 2019
Received in revised form 23rd May, 2019
Accepted 7th June, 2019
Published online 28th July, 2019

Key words:

Nano bubble, Carbon dioxide, Hydrogen, Reactive oxygen, Hydroxyl radical, Superoxide anion

ABSTRACT

Background/Aim: Nanobubble has a possibility to produce the hydroxyl radical by sonification, etc. The free radical such as hydroxyl radical is harmful for human beings. There is no research that has reported the effect of nanobubble to the free radicals such as hydroxyl radical and superoxide anion radical. In this research, the effect of nanobubble onpresence of free radicals is investigated.
Material & Method: N_2, O_2, H_2or CO_2 nanobubblesare produced by using a porous ceramic, whereas H_2 nanobubble is prepared by electrolysis. Hydroxyl radical is produced by H_2O_2solution irradiated by using ultraviolet light. Superoxide anion radicals are prepared by hypoxanthine/xanthine oxidase system. The radicals are measured by ESR using G-CYPMPOas trapping reagent.
Conclusion: CO_2 nanobubble can reduce the concentration of hydroxyl radical, while the CO_2 nanobubble increases the concentration of superoxide anion. H_2 nanobubble produced by electrolysis can reduce the superoxide anion concentration, however, the H_2 nanobubble cannot decrease the hydroxyl radical. The H_2 nanobubble produced in water containing CO_2 nanobubble is useful for scavenging both hydroxyl and superoxide anion radicals.

INTRODUCTION

Nanobubble has been applied in many fields such as wastewater treatment, medical application, food treatment and so on. For 20 years, many researches have reported regarding the quality and application of nanobubbles. The interesting points are stability of nanobubble and the possibility of radical producitons[1]. Several reports, which indicate that nanobubbles produced free radical and some others related on the free radicals are listed in Table 1. The produced or investigated free radical species, radical measurement method, nanobubble preparation method, type of gas in bubble, particle size measurement method and mean bubble size are compared. Most paper explained the hydroxyl radical production using nanobubbles, which size is about 100 to 200 nm. The radical production by ultrasonic wave irradiation becomes more important to produce the radicals, especially hydroxyl radical in water by comparing no irradiation and irradiation[4,5]. The aim of our report is not the production of radicals by nanobubbles, but how to eliminate the existing free radicals by nanobubbles.

Corresponding author: **Yuezhou Wei**
College of Resources, Environment and Materials, Guangxi University, China

The free radical produced Fenton reaction and ozone gas that can be decompose organic molecules, however, the free racial is toxic for human beings. Free radical is an atomic and molecular species with unpaired electrons and thus is paramagnetic. Free radicals are highly chemically reactive and usually short-lived because of those unpaired electrons, and thus have a strong oxidation capacity. Reactive oxygen species (ROS) belong to a heterogeneous group of highly reactive ions and molecules derived from molecular oxygen, among of which are free radicals as well as non-radical molecules such as hydrogen peroxide (H_2O_2)[6.] Some typical free radicals of ROS include hydroxyl radical (·OH), superoxide anion radical (·O_2^-), carboxyl radical (ROO·), nitric oxide radical (NO·) and peroxynitrite radical (·$ONOO^-$) [7]. ROS plays a crucial role in biology because it has been recognized as an important factor of oxidative damage in cells and tissues and cause of disaster, even cancer[8].Among all known ROS, hydroxyl radical and superoxide anion radical are two free radicals which have been discussed a lot inliterature. The majority of detrimental effects associated with lethal reperfusion injury are attributed to •OH[9]. Superoxide anion radical (·O_2^-) is a major by-product of aerobic metabolism and is a precursor to more toxic species [10]. In this work the elimination of hydroxyl radical and superoxide radical as the target free radicals has been investigated by using different kinds of nanobubbles. Nano-sized bubbles can stay in liquid for a long period and the specific surface area of nanobubble is extremely huge compared with ordinary bubble which leads to high surface tension, and also makes nanobubbles have high negative zeta potential in neutral solution. In this study, nanobubble dissolved water is investigated as a possible antioxidant which has scavenging capacity due to the presence of hydroxyl radical and superoxide anion radical.

Table 1 Reports regarding the relationship between free radical and nanobubble.

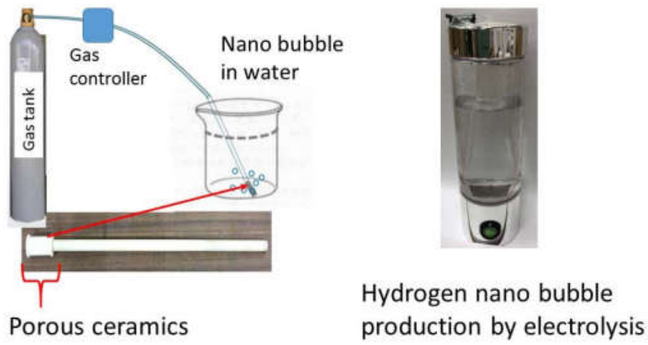
	Free radical Spices	Radical measurement method	Nano bubble Preparation method	Gas in nano bubble	Particle size measurement	Bubble size 50% (nm)
Takahashi et al. [2]	Hydroxyl radical generation	ESR +DMPO	Mechanical high speed agitation	Air	Particle counting spectrometer	10,000
Liu et al. [3]	Hydroxyl radical generation	Fluorescence with probe	Pressurized dissolution	O_2	Particle trajectory	60-180
Tada et al. [4]	Hydroxyl radical generation by sonication	ESR +DMPO	Two phase flow swirl system	Air	Particle trajectory	150
Ahmed et al. [5]	Hydroxyl radical generation by sonication	Fluorescence with probe	Porous media	Air, O_2, N_2, CO_2	Dynamic light scattering (DLS)	160-340
Our report	Effect on existing Hydroxyl radical & Super oxide	ESR + G-CYPMPO	Porous media & Electrolysis	H_2, O_2, N_2, CO_2, H_2+Ar	Interactive force apparatus (IFA)	115-180

MATERIALS & METHODS

Preparation of nanobubble dissolved water

Nano-sized bubbles indeionized water are generated by a pore type porous ceramic sparger, which has 500nm of mean pore diameter. Various gasesareinjected through this porous type sparger for 30 minutes at 0.4L/minutes in a beaker containing200mL water, while stirring using a magnetic stirrer as shown in Figure 1 (a). Nanobubbles are dispersed in water by injecting oxygen (O_2, 99.9%), nitrogen (N_2, 99.9%), carbon dioxide (CO_2, 99.5%) or 4% hydrogen (H_2) in argon gas. Also, the water containing hydrogen (EH_2) produced by electrolysis (Double hydrogen bottle, Woo Co., Ltd.) as shown in Figure 1(b) is employed. Hydrogen nanobubble is produced for 30 minutes in 200mL water. H_2 bubble concentration by electrolysis is 0.15vol%, determined by using a pycnometer. The nanobubble mixed solution of carbon dioxide and hydrogen are prepared by CO_2 bubbling for 30 minutes into the water containing hydrogen nanobubble produced by electrolysis for 30 minutes (CO_2 in EH_2) or electrolysis for 30 minutes to prepare hydrogen nanobubble into the water containing CO_2 by bubbling for 30 minutes (EH_2 in CO_2)

The prepared nanobubble size distribution in water is measured by interactive force apparatus (IFA) [11,12] that is well agreed with the dynamic light scattering method (DLS).



(a) (b)

Figure 1 Nanobubble production methods: (a) is porous ceramics spargermethod. (b) is electrolysis method.

ESR measurement

In this study, a novel radical trapper, 2-(5,5-dimethyl-2-oxo-2-l5- [1,3,2]dioxaphosphinan-2-yl)-2-methyl-3,4-dihydro-2H-pyrro- line N-oxide {2-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinan-2- yl)-3,4-dihydro-2-methyl-2H-pyrrole N-oxide, G-CYPMPO}, is used to trap free radical[13].A 25 mg of G-CYPMPO® (100 mM) is dissolved in 2 mL ultrapure water. A JEOL JES-TE25X ESR spectrometer (Tsukuba, Japan) is used to record ESR spectra of spin adducts. Typical ESR measurement conditions are as follow: microwave power, 4 mW; microwave frequency, 9.2 GHz; magnetic field, 328.0 mT; field sweep with, ±7.5 mT; field modulation, 0.16 mT; sweep time, 1 min; 0.003663 mT/Point, 4096 points in total. ESR measurements were performed at room temperature.

Water containing hydroxyl radical and superoxide anion radical

Hydroxyl radical is generated by the decomposition of 0.1 w/t% hydrogen peroxide under 5s UV-irradiation with a UV-irradiator (SUPERCURE-203S UV LIGHTSOURCE, Tsukuba, Japan). A mixture of 90 µL of 0.2 w/t% hydrogen peroxide and 90 µL of nanobubble included sample solution and 20µL of 100 mM G-CYPMPO is transferred into a disposable borosilicate ESR cell and ESR spectra of hydroxyl radical and G-CYPMPO adduct are analyzed.

Superoxide radical is generated in a hypoxanthine/xanthine oxidase (HX/XO) system. A mixture of 3.6 µL of 10.970 units/mL XO, 20 µL of 20 mM HX, 156.4 µL of nanobubble includedsample solution and 20 µL of 100 mM G-CYPMPO is transferred into a disposable borosilicate ESR cell and ESR spectra of superoxide radical and G-CYPMPO adduct are analyzed.

Kohri's ESR Spin-trap Method

A typical method, Kohri's ESR Spin-trap Method is used to analyze our data[14]. The peak-to-peak intensity of the selected ESR line of the free radical adduct was monitored in the presence or absence of antioxidant.

In the presence of the spin trap (ST) and antioxidant (AO), the following free radical (R) trapping reaction should occur:

R + Spin Trap → R-adduct rate constant : tk_{st}(1)
R + Antioxidant → product rate constant : k_{AO}(2)

When I_0 and I are the ESR peak height in the presence of ST alone and ST+Antioxidant, respectively, the amount of the product in equation (2) is I_0 - I. Thus, I_0 / I -1 are calculated to quantify free radical scavenging capacity.

To quantify free radical generation capacity, oxidant species are mixed with free radical generation system few hours before the ESR measurement. When I_0 and I are the ESR peak height in the presence of ST alone and ST+oxidant, respectively, the amount of free radical generation system oxidized by oxidant species is I_0 - I. Thus, I_0 / I -1 are calculated to quantify free radical generation capacity.

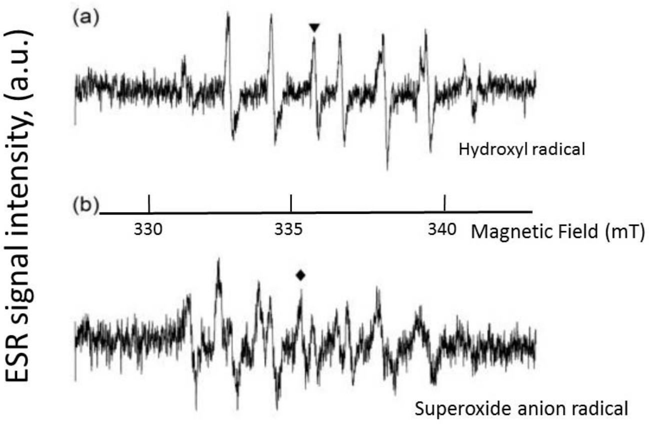


Figure 2 ESR spectra spin adducts of G-CYPMPO for (a) hydrogen peroxide solution (0.1w/v %) under 5s UV-illumination (b) Hypoxanthine/ xanthine oxidase (HX/XO) system. Diamond sign (◆) can indicate superoxide radical adduct, and inverted triangle sign (▼) can indicate hydroxyl radical adduct. The peak-to-peak intensity of the line marked with (◆) and (▼) was adopted as I_0 or I.

Figure 2(a) shows the ESR spectra of G-CYPMPO-trapped adduct in UV-illuminated hydrogen peroxide solution (0.1 w/v%). Figure 2(b) shows the ESR spectra of G-CYPMPO-trapped adduct in HX/XO system. Based on research of Oka T. etc., it is clear that Figure 2(a) is a typical eight-peak ESR spectra of hydroxyl radical G-CYPMPO-adduct[13]. Although Figure 2(b) is not a typical signal of superoxide radical G-CYPMPO-adduct, it can be regarded as a combination of hydroxyl radical and superoxide radical adduct signals. In this study, we focus only on the forth peak near the center of spectra (marked with diamond sign and inverted triangle sign) to quantify the amount of hydroxyl radical adduct and superoxide radical adduct.

RESULTS &DISCUSSION

Prepared nanobubblesize distribution

The size distributions of prepared various nanobubblesin water are shown in Figure 3. The 50% of mean diameters of N_2, O_2, H_2 and CO_2 bubbles are 180, 150, 130 and115nm, respectively. The N_2 bubble that did not react to water showed the largest nanobubble size, while the carbon dioxide bubble reaction with water is the smallest. The mean diameter of H_2 and argon mixture bubble size is almost same of H_2 bubble size produced by electrolysis.

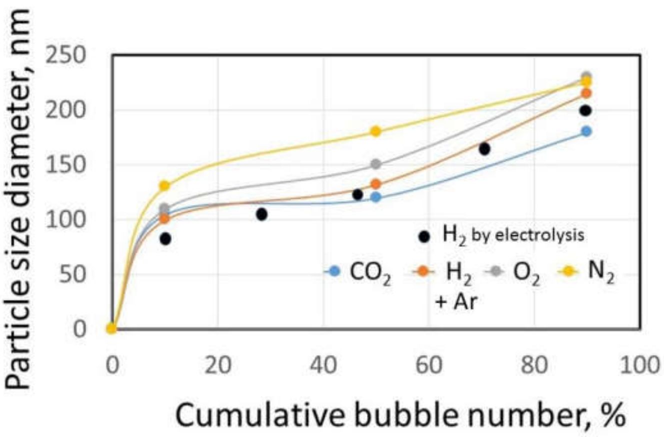


Figure 3 Comparison between the different fine bubble size distribution after 6 hours by blowing CO_2, H_2+Ar, O_2 and N_2 gas for 30minutesand H_2 produced by electrolysis method for 30 minutes.

After 4 days, the mean diameter of N_2 bubble is reduced to 130nm and remained the same even after 40 days. The mean diameter of H_2 bubbles becomes gradually smaller 50 nm after 4 days and 20nm after 40 days. On the other hand, CO_2 bubble is unstable and disappeared after several days at 1 atmosphere.

Free radical scavenging capacity by each nanobubbles gas in water

The free radical scavenging capacity of nanobubbles in water has been tested by ESR measurement. Figure 4 shows the result of I_0/I -1 derived from ESR spectrum of hydroxyl radical G-CYPMPO adduct in nanobubbles gas in water. Nanobubbles of carbon dioxide in water extremely shows the scavenging of hydroxyl radical. Comparing 6hours and 24 hours, the hydroxyl radical elimination decreases as the time passed for the decrease of CO_2 amount.

Although not as good as CO_2, $H_2$by electrolysis (EH_2) water,H_2, N_2 and O_2 can eliminate hydroxyl radical in a small amount and this capacity virtually was not influenced by elapsed time forgeneratingnanobubbles (i.e. from 6 to 24hours).

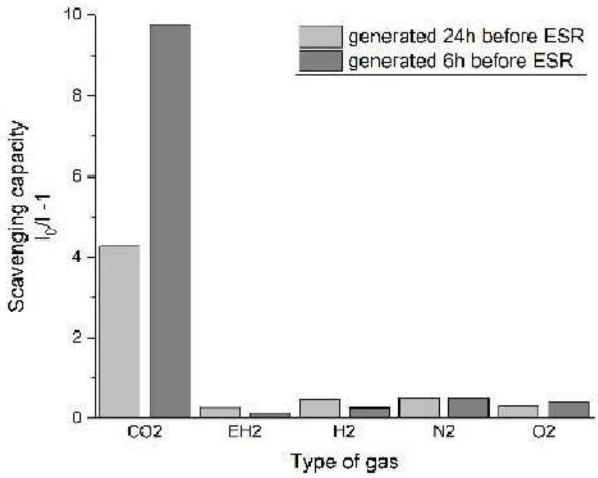


Figure 4 Plot of I_0/I - 1, where $I_0$and I denote ESR intensity in the absence and presence of nanobubble as hydroxyl radical scavenger. The value of I_0/I - 1 is the average of more than 5 times measurements.

Figure 5 shows the result of I_0/I -1 derived from ESR spectrum of superoxide anion radical G-CYPMPO adduct in nanobubbles gas in water. The H_2 by electrolysis (EH_2) water shows excellent performance in scavenging superoxide anion radical from 6 to 24 hours. Also H_2, N_2 and O_2 showed good scavenging capacity of superoxide anion radical. ForH_2, N_2 and O_2, superoxide anion radical scavenging capacity of the samples generated 6 hours before ESR is stronger than that of the samples generated 24 hours before ESR. On the other hand, CO_2 showed negative effect on scavenging superoxide anion radical. CO_2 nanobubble might produce superoxide anion radical after 6 to 24 hours.

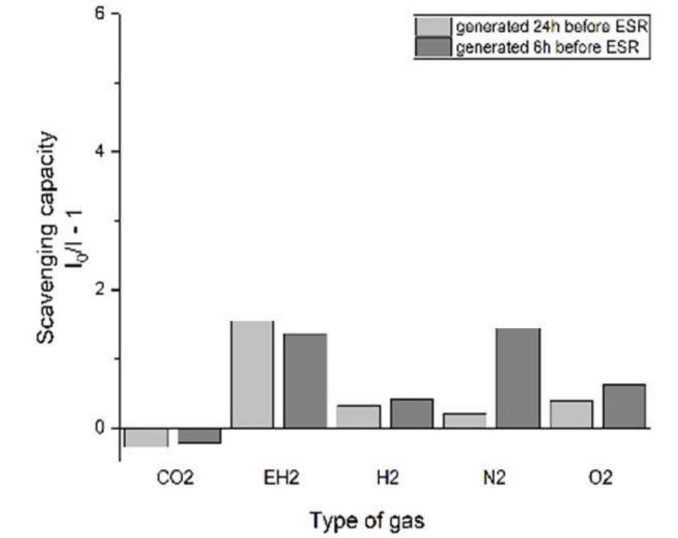


Figure 5 Plot of I_0/I - 1, where $I_0$and I denote ESR intensity in the absence and presence of nanobubble as superoxide anionradical scavenger. The valueof I_0/I - 1 is the average of more than 5 times measurements.

Free radical scavenging capacity by mixed nanobubbles gas in water

Hydroxyl radical scavenging is studied by introducing various types if nanobubble and the resultsare shown in Figure 6. After being prepared, nanobubble have been kept for 6 or 24 hours, before ESR spectrahave been measured and compared. A CO_2 bubble can reduce the hydroxyl radicals largest as shown in Figure 4. Next the EH_2 in CO_2 can reduce hydroxyl radical more than CO_2 in EH_2 by comparing 6and 24 hours. Only H_2 and EH_2 does not decrease the hydroxyl radical.

Superoxide anion radical scavenging is investigated by adding various nanobubble fluid and the result is shown in Figure 7. EH_2 shows the large decrease of peaks as shown in Figure 5. Also EH_2 in CO_2 can reduce the superoxide anion radical like EH_2. EH_2 in CO_2 shows better reduction than CO_2 in EH_2.

Therefore, EH_2 in CO_2 solution is the best water containing nanobubble to reduce both hydroxyl radical and superoxide anion radical.

CO_2 reacts to the hydroxyl radical as shown in Eq.(3) and produces ・CO_3H . Next through the reactions from (4) to (6), the hydroxyl radical is decomposed.

$$HO\cdot + O{=}C{=}O \rightarrow \cdot CO_3H \quad (3)$$

$$\cdot CO_3H + H_2O \rightarrow H\text{-}CO_3H + OH^- \quad (4)$$

$$H_2CO_3 \Leftrightarrow HCO_3^{2-} + H^+ \quad (5)$$

$$H^+ + OH^- \rightarrow H_2O \quad (6)$$

On the other hand, H_2 reacts to the superoxide anion radical as shown in equation (7) and produces hydroxyl radical.

$$\cdot O^-_2 + H_2 \rightarrow 2\cdot OH \quad (7)$$

The produced hydroxyl radical reacts to CO_2 and is decomposed.

The 100nm size of nanobubble moves by Brownian motion and the large specific surface area of gas bubble causes the rapid reaction. The water produced H_2 nanobubble in water containing CO_2 nanobubble is useful to scavenge both hydroxyl radical and superoxide anion radical.

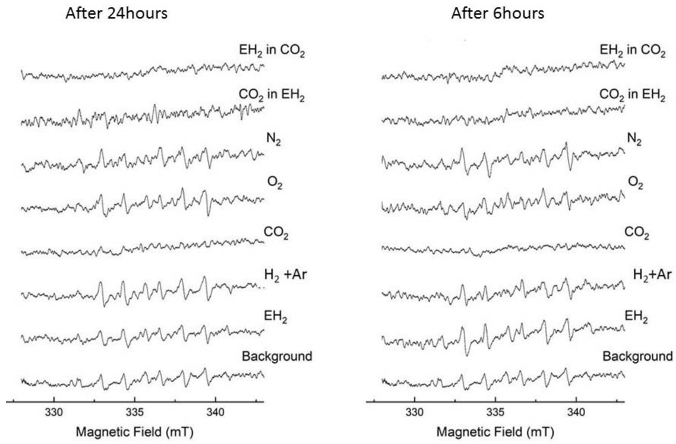


Figure 6 ESR spectra spin adducts of G-CYPMPO for hydrogen peroxide aqueous solution (0.1w/v %) containing various nanobubble gas under 5s UV-illumination

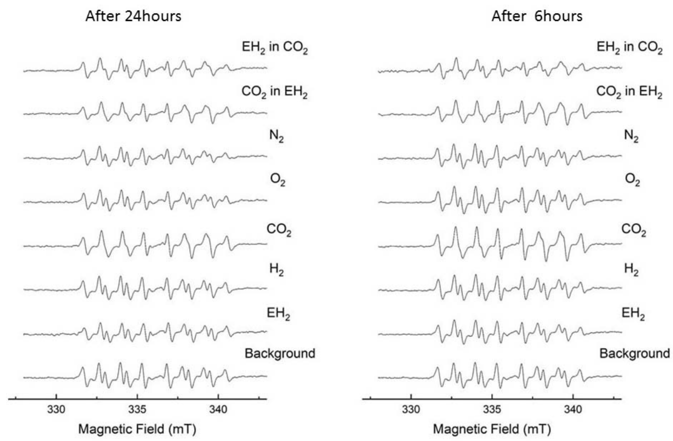


Figure 7 ESR spectra spin adducts of G-CYPMPO forhypoxanthine/ xanthine oxidase (HX/XO) aqueous solution containing various nanobubble gas.

CONCLUSION

The nanobubble of N_2, O_2, H_2 and CO_2were produced in water by porous ceramics, whereas H_2 is prepared by electrolysis. The average bubble size is from 115 to 180nm, and CO_2 bubble is the smallest. N_2 and H_2 nanobubble is stable for long time. The water containing nanobubble is added to the solution containing free radical of hydroxyl radical and superoxide anion radical. CO_2 nanobubble can reduce the hydroxyl radical, while the CO_2 nanobubble increases the superoxide anion concentration. H_2 nanobubble produced by electrolysis can reduce the superoxide anion concentration, however, the H_2 nanobubble cannot decrease the hydroxyl radical. However, the mixed fluid with H_2 and CO_2 can reduce the free radical. The water produced H_2 nanobubble in water containing CO_2 nanobubble is useful to scavenge both hydroxyl radical and superoxide anion radical.

Acknowledgement

The authors appreciate Mr. C.Suzuki and Mr. H.Abe for providing the hydrogen production bottle and M. Kamibayashi for supplingG-CYPMPO.

References

1. Yasui, K., Tuziuti T., Kanematsu W., Mysteries of bulk nanobubbles (ultrafine bubbles); Stability and radical formation, *Ultrasonics Sonochemistry,* (2018),doi: https://doi.org/10.1016/j.ultsonch.2018.05.038

2. Takahashi M., Chiba K., Li P., Free-Radical Generation from Collapsing Microbubbles in the Absence of a Dynamic Stimulus, *J. Phys. Chem. B*2007, 111, 1343-1347.
3. Liu S., Oshita O, Kawabata S., Makino Y., Yoshimoto T., Identification of ROS Produced by Nanobubbles and Their Positive and Negative Effects on Vegetable Seed Germination, *Langmuir,* 2016, 32, 11295−11302.
4. Tada K., Maeda M., Nishiuchi Y., Nagahara J., Hata T., Zhuowei Z., Yoshida Y., Watanabe S., Michio Ohmori M., ESR Measurement of Hydroxyl Radicals in Micro-nanobubble Water, *Chem. Lett.*, 2014, 43, 1907-1908.
5. Ahmed A.K.B., Shi X.,Likun Hua L.,Leidy Manzueta L.,Weihua Qing W., Marhaba T., Wen Zhang, W., *J. of Agricultural and Food Chemistry*, 2018 *66* (20), 5117-5124.
6. Galadari S., Rahman A., Pallichankandy S., Thayyullathil F., Reactive oxygen species and cancer paradox: to promote or to suppress?, *Free Radical Biology and Medicine* 2017, 104, 144-164.
7. Barry H., Reactive oxygen species in living systems: source, biochemistry, and role in human disease, *The American journal of medicine* 91.3, 1991, S14-S22.
8. Yoshimi S., Takemoto, T., Evaluation of scavenging rate constants of DOPA and tyrosine enantiomers against multiple reactive oxygen species and methyl radical as measured with ESR trapping method, *Bioorganic & medicinal chemistry letters*,25.8, 2015, 1808-1810.
9. Sano M., Multifaceted approach to analysing the pathogenesis of cardiovascular disease, *Circulation Journal* 76.11, 2012, 2521-2529.
10. James A. I., Cellular defenses against superoxide and hydrogen peroxide, *Annu. Rev. Biochem.* 77, 2008, 755-776.
11. Fujita T, Kurose T., Ponou J., Dodbiba G, Shibayama A., Otsuki A., Nano Bubble and Emulsion Size Distribution Measurement by Interactive Force Apparatus., *International Journal of the Society of Materials Engineering for Resources*, Vol. 23, 2018, No. 2, pp. 158 - 161.
12. Fujita T. , Dodbiba G., Okaya K., Matsuo S., WangL.P., Onda K. and Otsuki A., Nano to micro size distribution measurement in the fluid by interactive force apparatus for fine particle processing, *Journal of Nanoscience and Nanotechnology*, Vol.13, 2013, pp.8184-8189.
13. Oka, T., Midoricawa, M., Saiki, S., Muroya, Y., Kamibayasshi, M., Yamashita, M., Anzai, K. Spin-Trapping Reactions of a Novel Gauchetype Radical Trapper G-CYPMPO. *Anal. Chem.*2011, *83*, 9600-9604.
14. Kohri S., Fujii H., Oowada S., Endoh, N., Sueishi, Y., Kusakabe M., Shimmei M., Kotake, Y.,An oxygen radical absorbance capacity-like assay that directly quantifies the antioxidant's scavenging capacity against AAPH-derived free radicals, *Analytical biochemistry*, 386.2 ,2009, 167-171.

How to cite this article:

Toyohisa Fujita *et al* (2019) 'Nanobubble Mixed Solution of Carbon Dioxide And Hydrogen For Removinghydroxyl And Superoxide Anion Free Radicalsin Water ', *International Journal of Current Advanced Research,* 08(07), pp. 19438-19442. DOI: http://dx.doi.org/10.24327/ijcar.2019.19442.3750

****