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Technical Note

A Simple Method for Nanobubble Generation and Stability of the Bubbles

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1. Introduction

It has been reported that ultra-small gas bubbles in liquid with diameters of micron and submicron-order, so called micro-nanobubbles, have some characteristic physical and chemical properties. They show slow buoyancy, negative surface charges [19,22], free radical formation [12,20] and increased water molecule mobility [14,22]. These properties of micro-nanobubbles might cause miraculous effects on growth of plants [4,18] and fishes [4,17]. Application of these tiny bubbles for many fields of technology attracts great attention [1]. In the field of environmental biotechnology, miro-nanobubbles is applied for cleaning solid surface [13,23] and for waste water treatment, for example, degradation of noxious chemicals such as phenol [11]. Further, it is expecting to use micro-nanobubbles in medical field such as drug delivery [6] and tumor destruction [15]. The use of ozone nanobubble water as an adjunct to periodontal treatment is proposed [5].

However, basic study of micro-nanobubbles, particularly nanobubbles, remains still in the beginning. It has long been believed that nanobubbles cannot exist for a long period, since a high internal gas pressure of the bubbles drives gas diffusion across the interface resulting instantaneous dissolution of bubbles [2]. No fundamental research on the mechanism of biological effect of nanobubbles has been made. To conduct a laboratory experiment, we need to get nanobubbles that are stable in size and exist for long periods. Generation of micro-nanobubbles in water are mainly performed by two methods that are based on decompression and gas-water circulation [1]. Ultrasonic waves are also used to generate microbubbles through the cavitation of water [9,15]. A small-porous-glass membrane has been reported as a useful device for nanobubble formation [10]. Anyhow, a sophisticate device has been used to generate micro-nanobubbles, and bubble size and quantity were variable among each instrument.

In the present experiment, we have succeeded to generate air-nanobubbles easily using a household hand mixer. The size and stability of the air-nanobubbles were determined using a dark field microscope, an electron microscope, and a nanoparticle analyzer. It was concluded that most of the nanobubbles generated were smaller than 100 nm in diameter and existed for over one month. In the present report we use the term, “gas nanoparticle (GNP)”, instead of nanobubble.

2. Materials and methods

Pure water was prepared by Direct Q-UV (Millipore S.A.S, Molsheim, France) and the conductivity of water was higher than 18.5 MΩ·cm. The screw of the hand mixer (Braun MR 400plus, Braun Co. Germany) was dipped into 100 mL water in a 300 mL-beaker and the water was swirled for 5 to 30 min on ice. Since a swirling period of 30 min gave about ten times higher concentration of GNPs than that of 5 min, we adopted 30 min swirling to generate GNPs regularly. To prevent overheating of the motor, one swirling was shorter than 5 min, so that 6 motors were used for 30 min swirling.

The GNPs were observed with a dark field microscope (IX71-TIR, Olympus Co. Japan) equipped with epi-illumination device and high speed ECD camera. The GNPs were also observed by electron microscopy as reported previoously [21]. A field-emission gun-type TEM (JEOL Ltd., Tokyo, Japan, JEM-2010) was used to observe the replica film at a 200-kV acceleration voltage.

Numbers of the GNPs were measured by direct counting at 800×magnification. The polystyrene beads with diameters of 50 nm, 100 nm and 200 nm (Polysciences Inc., PA, U.S.A.) were used as standards for size and number. The size and zeta potential were measured by a nanoparticle analyzer based on dynamic light scattering (Zetasizer Nano, Malvern/Sysmex Co., England). Generally, this type of particle analyzer cannot measure the quantity of particles but shows size distribution in percentage only. Thus, we added a known amount of internal standard to the sample and roughly estimated the amount of GNP in the sample. Thus obtained values were well agreed with those using dark field microscopy and electron microscopy.

3. Results and discussion

No nanoparticle was detected in pure water when analyzing with a dark field microscope or nanoparticle analyzer, suggesting that contamination of other particles from air is negligible. When pure water was swirled for 30 min, GNP was generated with many different diameters, namely, 40 to 500 nm. The pattern of size distribution was variable in each experiment. Fig. 1A shows a typical pattern of the size distribution of GNP in pure water. As for quantity of GNP, several GNPs were observed by dark field microscopy at a magnification of 800. The numbers of GNPs were estimated roughly to be less than 3×10^8 mL^{-1}. The addition of glycerol to pure water at 1% before swirling did not increase the numbers of GNP.

When we added ethanol to pure water at 1% and swirled for 30 min, the GNP numbers increased to several times comparing those of pure water (Data not shown). The addition of ethanol at 2% slightly enhanced GNP generation than that at 1% ethanol. However, the addition of ethanol at 3% decreased the numbers of GNP to less than 70% comparing with those at 1% ethanol. The addition of ethanol to pure water at 10% did not enhance GNP generation. Since we are attempting to apply GNP for bacterial growth regulation, and 2% ethanol is toxic enough for the growth of ordinal microorganisms, we adopted ethanol concentration of 1% to generate GNPs.

Fig. 1B shows the size distribution pattern of GNPs in 1% ethanol, in which a sharp peak appears at about 40 nm diameter. On rare occasions, a very small gentle peak appears at about 160 nm diameter. No GNP or contaminated particle, larger than 500 nm diameter, was observed. In the presence of 1% ethanol, the peaks in diameter were fixed around 40 nm for over one month (Data not shown). It is concluded that the size of GNPs was stabilized in a diameter smaller than 100 nm by the addition of ethanol at 1%. The length of swirling period did not affect stability of the size of GNPs, namely, GNP concentration would not affect its stability.

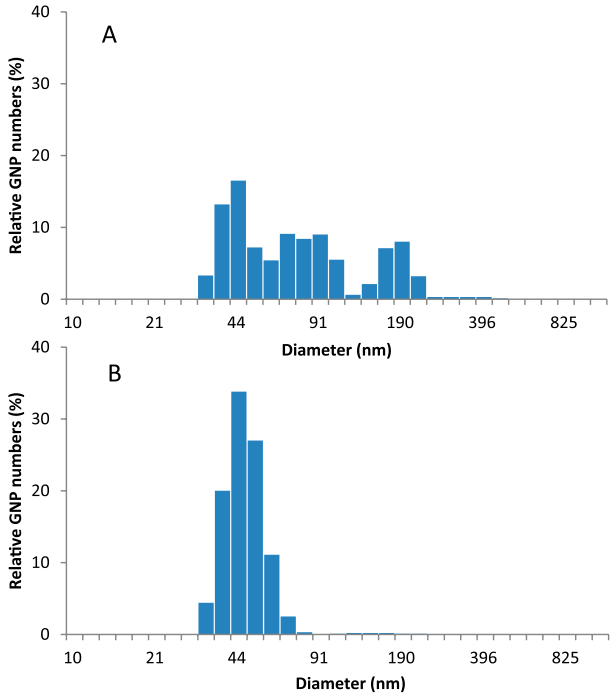

Fig. 1. Size distribution of GNPs
GNPs were generated in pure water (A) or in 1% ethanol (B). Size of GNPs was measured using Zetasizer Nano. Vertical in the figure shows relative numbers of GNP (%) of each size to total GNP numbers.

Fig. 2 shows the change in numbers of GNP in the presence of 1% ethanol when the GNP water was stored in refrigerator for about 2 months. It is shown that the numbers of GNP (1 to 2×10^9 per mL) were kept unchanged for over two months; that is, once formed GNP in small sizes were present in water stably so long periods as reported previously [16]. It has been reported that the life time of bubbles with mean bubble radii of less than 1 μm becomes over one year when amphiphilic molecules crystallize on the air-liquid interface [3].

Microbubbles have been reported to be negatively charged with a zeta potential about –30 mV [19], and became more negative with increasing pH [9]. It has also been reported that nanobubbles formed in α-cyclodextrin aqueous solution are unstable in a higher ionic strength [7]. We determined zeta potential of the GNPs and the effect of NaCl on zeta potential. The pH of the GNP water was about 6.5. In the absence of NaCl, the average zeta potential was -32.9 ± 3.0 mV. When NaCl was added at concentrations of 0.002% and 0.5%, the zeta potential became -16.0 ± 1.6 and -5.8 ± 0.3 mV, respectively. The addition of NaCl seemed to cancel out the negative charge of GNP. It is speculated that Na^+ might be attracted to the negatively charged surface of GNP, and which might cancel out the negative charge of the GNP.

Fig. 3 shows the effect of NaCl on the size of GNP. The addition of NaCl up to 0.3% did not significantly affect the size of GNPs; that is, the diameters were kept smaller than 100 nm. However, the diameter increased to 190 nm in 1% NaCl, and to 600 nm in 5% NaCl. These results were well agreed with the observation using a dark field microscope, where the GNPs disappeared by adding 5% NaCl. It is considered that 10 times increase in diameter results in 1,000

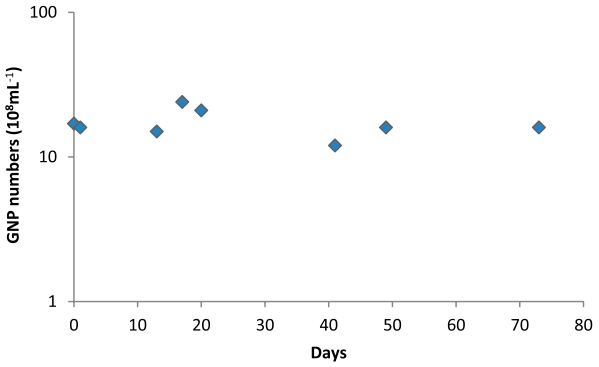

Fig. 2. Durability of GNPs
GNPs in 1% ethanol were stored at 4°C. Numbers of GNPs were measured using Zetasizer Nano.

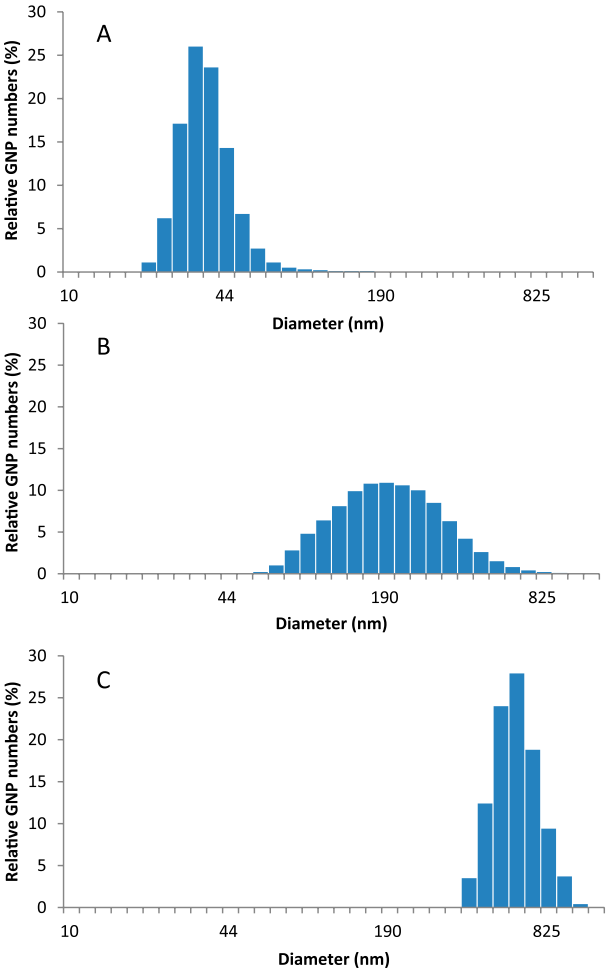

Fig. 3. Effect of NaCl on the size of GNPs
(A): without the addition of NaCl. (B): NaCl was added at 1%. (C): NaCl was added at 5%. Size of GNPs was measured using Zetasizer Nano. Vertical in the figure shows relative numbers of GNP(%) of each size to total GNP numbers.

times increase in the volume of GNPs; that means, 1,000 small GNPs disappears when one big GNP with ten times larger diameter formed. Under the circumstances, we can unlikely detect GNPs in the field of microscope (800×). The decrease in negative zeta potential might result in coalescence of GNPs, which make the size of GNPs larger [8]. Considering these matters, to produce small GNPs in sea water will be very difficult.

Why 40 nm size GNP was predominant in 1% ethanol, is a very difficult question. It has been proposed that the addition of a small amount of propanol affect the hydrogen-bonding network at the gas-water interface [19]. A certain thermodynamic situation may determine the size and quantity of GNPs. Other physicochemical properties should be revealed to know real nature of GNPs, and to use GNPs more effectively in biotechnology. Precise estimation of GNP numbers is still a matter of problem. We need a reliable standard assay technique for GNP counting. We are now going to elucidate the mechanism of the effect of GNP on biological processes using the water rich in GNPs.

4. Conclusion

We could generate very small air-nanobubbles, having diameters smaller than 100 nm, using a household hand mixer. The bubbles were stable in water containing 1% ethanol more than one month and show zeta potential of –30 to –40 mV. The addition of NaCl to nonobubble-water caused changes in the diameter and the number of bubbles; larger in size and lesser in numbers.

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