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FREE RADICAL FORMATION BY ULTRASOUND IN AQUEOUS SOLUTIONS. A SPIN TRAPPING STUDY

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Our recent spin trapping studies of free radical generation by ultrasound in aqueous solutions are reviewed. The very high temperatures and pressures induced by acoustic cavitation in collapsing gas bubbles in aqueous solutions exposed to ultrasound lead to the thermal dissociation of water vapor into H atoms and OH radicals. Their formation has been confirmed by spin trapping. Sonochemical reactions occur in the gas phase (pyrolysis reactions), in the gas-liquid interfacial region, and in the bulk of the solution (radiation-chemistry reactions). The high temperature gradients in the interfacial regions lead to pyrolysis products from non-volatile solutes present at sufficiently high concentrations. The sonochemically generated radicals from carboxylic acids, amino acids, dipeptides, sugars, pyrimidine bases, nucleosides and nucleotides were identified by spin trapping with the non-volatile spin trap 3,5-dibromo-2,6-dideuterio-4-nitrosobenzenesulfonate. At low concentrations of the non-volatile solutes, the spin-trapped radicals produced by sonolysis are due to H atom and OH radical reactions. At higher concentrations of these non-volatile solutes, sonolysis leads to the formation of additional radicals due to pyrolysis processes (typically methyl radicals). A preferred localization of non-volatile surfactants (compared to analogous non-surfactant solutes) was demonstrated by the detection of pyrolysis radicals at 500-fold lower concentrations. Pyrolysis radicals were also found in the sonolysis of aqueous solutions containing only certain nitrone spin traps. The more hydrophobic the spin trap, the lower the concentration at which the pyrolysis radicals can be observed. The effect of varying the temperature of collapsing transient cavities in aqueous solutions of different rare gases and of N_2O on radical yields and on cell lysis of mammalian cells was investigated.

KEY WORDS: Ultrasound, spin trapping, electron spin resonance, transient cavitation, pyrolysis radicals, surfactants.

INTRODUCTION

Ultrasound is widely used for diagnosis and therapy in medicine, and therefore the potentially damaging effects of transient cavitation are of interest. In 1982, theoretical studies by Flynn[1] and Apfel[2] predicted that for certain exposure conditions transient cavitation is possible for microsecond pulses of 1 MHz ultrasound. This prediction was confirmed by electron spin resonance (ESR) and spin trapping[3] and subsequently by chemiluminescence[4] experiments.

Acoustic cavitation involves the formation, growth and collapse of small gas bubbles in liquids exposed to ultrasound.[5,6] These processes effectively convert the low-energy density of a sound field into the high-energy density in the interior and surrounding of collapsing gas bubbles.

Cavitation can be divided into two types, stable and transient. Stable cavitation

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bubbles oscillate radially about an equilibrium size and persist for many cycles. At frequencies of 1 MHz and 50 kHz, the resonance radii of argon bubbles in water are approximately 4 μm and 80 μm, respectively.[7]

Transient cavitation bubbles, on the other hand, exist for only one or two acoustic cycles and after expanding to two to three times their resonance size during the negative acoustic pressure half-cycles, they collapse violently during a single compression half-cycle. During the final stage of collapse, the speed of the bubble wall reaches at least the speed of sound in the liquid, and the temperature and pressure in the gas bubble reaches thousands of degrees K and hundreds of atmospheres,[5,6] respectively.

By measurements of the rates of CO formation from a series of metallocarbonyls [(Cr(CO)$_6$, Mo(CO)$_6$, Fe(CP)$_6$, and W(CO)$_6$] in the sonolysis of argon-saturated liquid alkanes, Suslick *et al.*[8,9] succeeded in experimentally measuring the temperature of the gas phase reaction zone of collapsing cavitation bubbles (5200 ± 650 K) and the temperature of the surrounding liquid shell (200 nm) was calculated to be 1900 K with a lifetime of 2 μ sec.

For adiabatic collapse of a cavitation bubble,[10,11] the final intracavity temperature at the end of the collapse, T_{fi} is given by

$$T_{fi} = T_{in}(R_{max}/R_{min})^{3(\gamma - 1)}$$

where T_{in} is the initial temperature, γ is the specific heat ratio (C_p/C_v) of the gas inside the bubble, R_{max} is the initial radius of a bubble which collapses to a final radius of R_{min}.

From the energy equation, Noltingk and Neppiras[10,11] found

$$(R_{max}/R_{min})^{3(\gamma - 1)} = P(\gamma - 1)/P_{in}$$

where P is the external pressure (the sum of the hydrostatic and acoustic pressures) and p_{in} is the inital pressure of non-condensible gas inside the cavity of radius R_{max}.

Hence,

$$T_{fi} = T_{in}\,[P\,(\gamma - 1)/p_{in}]$$

For monatomic gases with $\gamma = 1.67$, $T_{in} = 300$ K and $P/p_{in} = 15$, the final collapse temperature T_{fi} is 3015 K while for the triatomic gas N$_2$O, $\gamma = 1.30$, $T_{in} = 300$ K, and $P/p_{in} = 15$, it follows that $T_{fi} = 1350$. This illustrates the importance of γ in determining the collapse temperature.

Sonoluminescence is a weak emission of light observed when ultrasound above a certain intensity is passed through a liquid containing dissolved gases.[12] Sonoluminescence studies of rare gases in aqueous solutions have shown that the emission intensity increased with decreasing thermal conductivity of the gas.[13,14] This indicates that the collapse of cavitation bubbles is not completely adiabatic and can be explained by the loss of heat from the bubble to the surrounding liquid due to the high temperature gradient at the gas-liquid interface.[13,14]

Young[14] estimated the final collapse temperatures for aqueous solutions saturated with helium, neon, argon, krypton, and xenon to be 815, 1420, 1650, 1890, and 2000 K, respectively. Although many simplifying assumptions were used in this calculation, it can still be used as a qualitative guide.

Nevertheless, a more detailed understanding of sonochemical reaction mechanisms can be obtained by changing the "effective" temperature of transient cavitation bubbles in order to separate the contributions of individual reaction steps with widely different energies of activation, such as those requiring the breaking of oxygen--hydrogen and carbon–carbon bonds.

In the last few years it has become clear that sonochemical reactions can occur in three different regions.[15] The first region is the gas phase of collapsing gas bubbles. In this region hydrogen atoms and hydroxyl radicals are formed by the thermal dissociation of water. Any volatile organic solute in this region will also participate in combustion processes, often giving rise to radicals typical of pyrolysis.[16] The second region is the interface between the hot gas bubbles and the bulk of the solution where large gradients of temperature and pressure as well as high radical concentrations exist. Non-volatile solutes containing charged groups to anchor them in the aqueous phase may also give rise to pyrolysis radicals provided they are present at high concentrations in the interfacial region.[17]

The third region is the bulk solution at ambient temperature, where the observed products are similar to those observed in aqueous radiation chemistry and where radical scavenging reactions follow the kinetics observed in gamma-radiolysis.[18]

SPIN TRAPPING STUDIES OF AQUEOUS SONOCHEMISTRY

In the spin trapping method,[19] the short-lived free radicals react with a diamagnetic nitroso or nitrone spin trap to produce a longer lived free radical which can be detected and often unambiguously identified by conventional ESR. Recently, we have found that 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), which is a water-soluble, nonvolatile, aromatic nitroso spin trap, was useful for the detection of sonochemically induced radicals.[20] The sulfonate group insures nonvolatility and high solubility, and several of the carbon-centered radical adducts show sufficiently detailed spectra to allow identification of the trapped radicals.[20]

Our initial ESR and spin trapping studies[21,22] were carried out in aqueous argon-saturated solutions exposed to 50 kHz ultrasound. The spin traps 5,5-dimethyl-l-pyrroline-N-oxide (DMPO) and 4-(N-methylpyridinium)-t-butylnitrone (PYBN) were used to detect OH spin adducts and the H spin adducts were observed with DMPO, PYBN and α-(4-pyridyl-l-oxide)-N-t-butylnitrone (POBN). In D_2O solutions with the latter spin traps, the D spin adducts were observed. By studying the competition reactions for OH and H between the spin traps DMPO and POBN and a large variety of H and OH scavengers [formate, thiocyanate, benzoate, methanol, ethanol, l-propanol, 2-methyl-2-propanol, acetone, and 2-methyl-2-nitrosopropane (MNP)] further confirmation for the formation of OH radical and H atoms by ultrasound was obtained.[22]

From plots of DMPO-OH yields vs. $k_{\text{scavenger + OH}}$[scavenger] and of POBN–H yields vs. $k_{\text{scavenger + H}}$ [scavenger], it was shown that certain volatile scavengers (acetone, MNP) were two orders of magnitude more effective than predicted from their rate constants in homogeneous solution, consistent with scavenging processes either in the gas phase or in the interfacial regions, or both.[22]

Henglein and Kormann[23] measured the hydrogen peroxide yields in the sonication of argon-saturated water in the presence of various volatile solutes. The $C_{1/2}$ value of a scavenger is defined as the scavenger concentration which decreases the H_2O_2 yield to 50% of the yield observed in the absence of the scavenger. The efficiency of OH radical scavenging as measured by $C_{1/2}$ correlates closely with the hydrophobicity of the solutes; $C_{1/2}$ ranges over 4 orders of magnitude. The effects were explained by OH radical formation in the gas phase, recombination of OH radicals to produce H_2O_2 in the interfacial region, and preferential accumulation of the scavenger either at the interface or in the gas phase.

In order to distinguish between reactions in the interfacial region and those occurring in the hot gas bubbles, non-volatile surfactants were used to probe the interfacial regions of cavitation bubbles.[24] Hydrogen peroxide formation in the sonolysis of argon-saturated solutions of surfactants [sodium dodecyl sulfate (SDS), dodecyltrimethylammonium bromide, and n-octyl-beta-D-glucopyranoside] was markedly decreased compared to solutions containing the corresponding non-surfactants such as sodium acetate (NaAc) and tetramethyl-ammonium bromide. The $C_{1/2}$ value for sodium acetate is 1 M while that for sodium dodecyl sulfate is 1 mM. However, the ratio of the rate constants for the reaction of SDS and NaAc with OH is 76, suggesting that the larger efficiency of scavenging OH radicals by SDS is due to a preferential localization of the surfactant in the interfacial region. This was confirmed by the detection of pyrolysis-derived methyl radical adducts of 3,5-dibromo-2,4-dideuterio-4-nitrosobenzenesulfonate (DBNBS-d_2) at a 500 times lower concentration of the surfactants n-octyl-beta-D-glucopyranoside (OGP) and n-decyl-beta-D-glucopyranoside (DGP) than for the non-surfactant analogue methyl-beta-*D*-glucopyranoside (MGP).

The secondary carbon radicals produced from OGP and DGP by H and OH radical abstraction reactions were detected at much lower solute concentrations (50 μM) than for MGP (50 mM), consistent with the preferential accumulation of surfactants at the gas liquid interface when 50 kHz ultrasound was used. It will be of considerable interest to explore whether this localization can also occur at higher frequencies when the time of the half cycle during which collapse of the cavitation bubble occurs is decreased (i.e., at 5 MHz the collapse time is 10^2 times less than at 50 kHz).

Previous studies have shown that the sonolysis of aqueous solutions of volatile solutes leads to free radicals formed by thermal decomposition and also to isotopic exchange in the gas phase.[16,25–27] In recent studies of sonolysis of dilute solutions of nonvolatile solutes such as acetate,[17] sodium propionate,[17] amino acids,[20] peptides,[20] pyrimidines,[28,29] nucleosides,[28] nucleotides, and sugars,[17] only radicals formed by hydroxyl radical and hydrogen atom reactions could be detected. The radicals formed by hydroxyl radical reactions were identified by comparison with the radicals produced by UV photolysis in the presence of hydrogen peroxide. For pyrimidines,[28,29] nucleosides,[28] and nucleotides, radicals formed by addition to the 5,6 pyrimidine double bond were identified.

However, at higher solute concentrations, additional new radicals (typically methyl radicals) formed in the interfacial high temperature regions induced by transient cavitation were found for sodium acetate,[17] sodium propionate,[17] amino acids,[17] sugars,[17] and nucleotides.

For all of the nonvolatile solutes, a similar dependence of radical yield on solute concentration was observed. The results for sodium acetate are shown in Figure 1. With increasing solute concentration, the yield of radicals formed by H and OH radical reactions (CH_2COO^-) increases at low solute concentration, reaches a maximum, and then decreases [Figure 1(b)]. The pyrolysis radical yield (CH_3) is observed at higher solute concentrations, and is initially directly proportional to solute concentration over a certain range [Inset of Figure 1(a)] and continues to increase in the region where the yield of radicals produced by OH and H radicals reaches a maximum and then decreases [Figure 1(a)]. [At the very high solute concentrations which can be

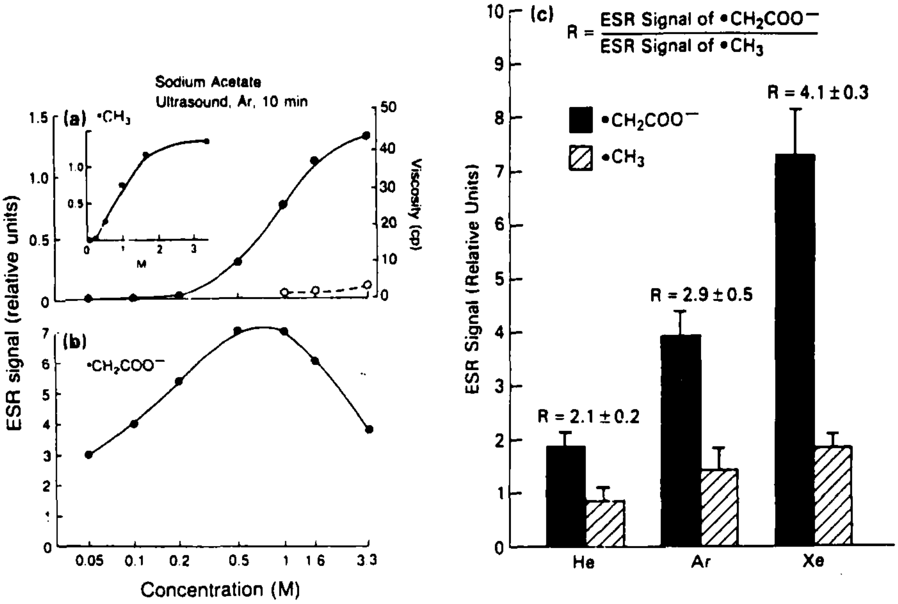

FIGURE 1(a,b) Effect of sodium acetate concentration on the relative radical yields of (a) $\dot{C}H_3$ radical and (b) $\dot{C}H_2COO^-$ radical. Dashed line shows viscosity of sodium acetate solution. Inset shows $\dot{C}H_3$ radical yield as a function of a linear concentration scale. (c) Effect of rare gases (He, Ar, Xe) on the formation of $\dot{C}H_3$ and $\dot{C}H_2COO^-$ radicals from sonicated aqueous solutions of sodium acetate (3.3 M, sonication time 10 min). From ref.[17].

attained with sugars the viscosity of the solution rises sharply and all radical yields decline due to decreasing cavitation (e.g., from 5 to 7 M 2-deoxy-D-ribose, the viscosity increases from 8.8 to 43.2 cP)].

The sonochemically induced decomposition of certain nitrone spin traps in aqueous solutions was investigated in the absence of other solutes.[30] When argon-saturated aqueous solutions of PBN were sonicated, the spin adducts of PBN-phenyl, PBN-H and an unidentified spin adduct were observed. In the presence of DBNBS-d_2 the DBNBS-d_2 spin adducts of methyl and t-butyl radicals from the pyrolysis of PBN were also generated. Similar spin adducts induced by pyrolysis were also found in sonicated aqueous solutions of other nitrone spin traps such as POBN and α-(4-nitrophenyl)-N-*tert*-butylnitrone. The greater the hydrophobicity of the spin trap, as determined by its 2-octanol/water partition coefficient, the lower the concentration of spin trap at which pyrolysis (methyl) radicals formed by thermal decomposition of the spin trap can be observed. The relatively non-volatile, highly hydrophobic spin traps accumulate preferentially in the interfacial region of cavitation bubbles where they undergo thermal decomposition to produce radicals. In 99.7% D_2O solutions of PBN or POBN large yields of PBN-H or POBN-H are formed from the spin traps or their decomposition products by homolytic scission of C–H bonds in addition to the PBN-D or POBN-D spin adducts which arise from D atoms from the thermal decomposition of D_2O vapor in the cavitation bubbles.[31]

In order to attain different final temperatures of collapsing cavitation bubbles, aqueous solutions of sodium acetate were saturated with He, Ar, and Xe during sonolysis.[17] The ESR signals of the spin adducts of $\dot{C}H_2COO^-$ and of $\dot{C}H_3$ were measured after 10 minutes of sonication at 50 kHz in the presence of the different rare gases and the ratio R = (ESR signal of $\dot{C}H_2COO^-$)/(ESR signal of $\dot{C}H_3$) was calculated. Both the radical yields and the ratio R increased with increasing final temperature and decreasing thermal conductivities of the rare gases [He = 0.3665, Ar = 0.0385, Xe = 0.0124 (cal/cm/s/°C × 10^3)].[14] This is illustrated in Figure 1(c).

The energy of activation for the thermal decomposition of water in the gas phase in the 2000–5000 K range is 478 kJ while for a typical C–C scission, such as the decomposition of ethane to methyl radicals in the 800–2500 K range, the recommended value is 285 kJ.[17] Similarly, the energy of activation for C–C scission of acetate in the interfacial regions can be expected to be much lower than that for the decomposition of water in the gas phase. Hence, increasing the final temperature of the collapsing cavitation bubbles would be expected to have a larger effect on the increase in OH radical and H atom formation, and consequently on the yield of $\dot{C}H_2COO^-$ radicals formed by abstraction reactions than on the yield of $\dot{C}H_3$ radicals formed by the thermal decomposition of acetate ions in the interfacial region. The ratio $R = [\dot{C}H_2COO^-]/[\dot{C}H_3]$ was found[17] to be 2.1 ± 0.2 for He, 2.9 ± 0.5 for Ar, and 4.1 ± 0.3 for Xe.

More generally, we found the following results.[31] If R_U is the sonolytic radical yield of the radical formed directly by a reaction or indirectly by a series of reactions with

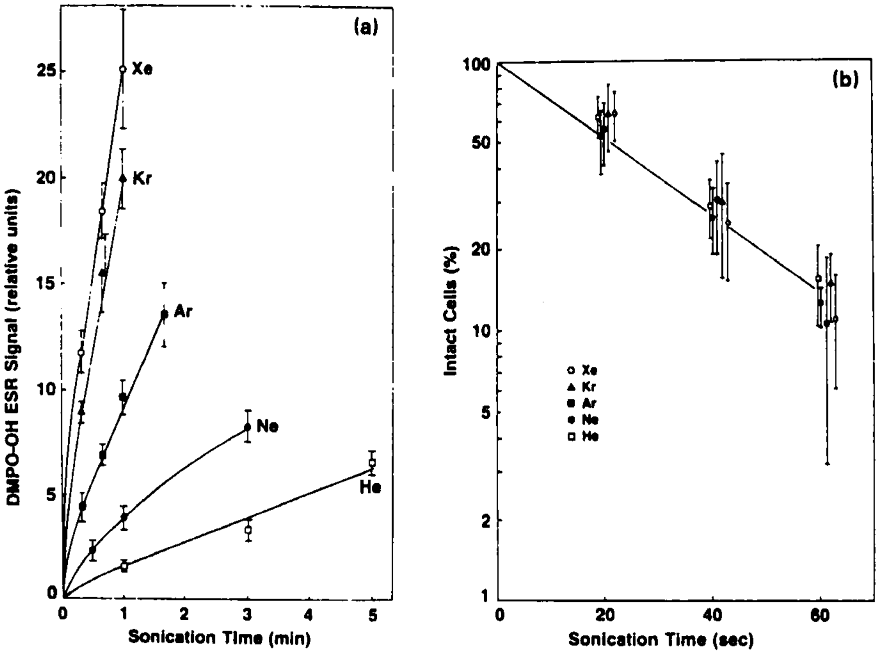

FIGURE 2(a) Sonication time-dependence of ESR signal intensity of the DMPO–OH adduct obtained in aqueous DMPO solutions bubbled with different rare gases. Bars indicate different standard deviations (three or four experiments). (b) The relationship between the fraction of intact CHO cells immediately after irradiation with 50 kHz ultrasound and the sonication time. Bars indicate standard deviations (three or four experiments). From ref.[32].

the higher energy of activation, and R_L is the radical yield produced by processes with the lower energy of activation, then the ratio R_U/R_L increases with increasing temperature of the collapsing cavitation bubbles (Xe > Kr > Ar > Ne > He) and decreasing thermal conductivity of the rare gas.

We have investigated the effect of varying the temperature of cavitation bubbles in aqueous solutions of different rare gases on the formation of hydroxyl radicals by 50 kHz ultrasound with DMPO as the spin trap.[32] In Figure 2(a) one unit of the Y-axis corresponds to 5.5×10^{-8} mol dm^{-3} of DMPO-OH adduct by comparison with a standard nitroxide. The DMPO-OH yields were in the order Xe > Kr > Ar > Ne > He, in accord with the higher temperatures of the cavitation bubbles and consistent with the effect of the thermal conductivity of the rare gases on sonoluminescence [Figure 2(a)]. However, cell lysis of Chinese hamster ovary cells was the same for all of the rare gases and independent of their thermal conductivity and the "effective" temperatures of the cavitation bubbles [Figure 2(b)].

These experimental results can be understood in terms of a mathematical model of

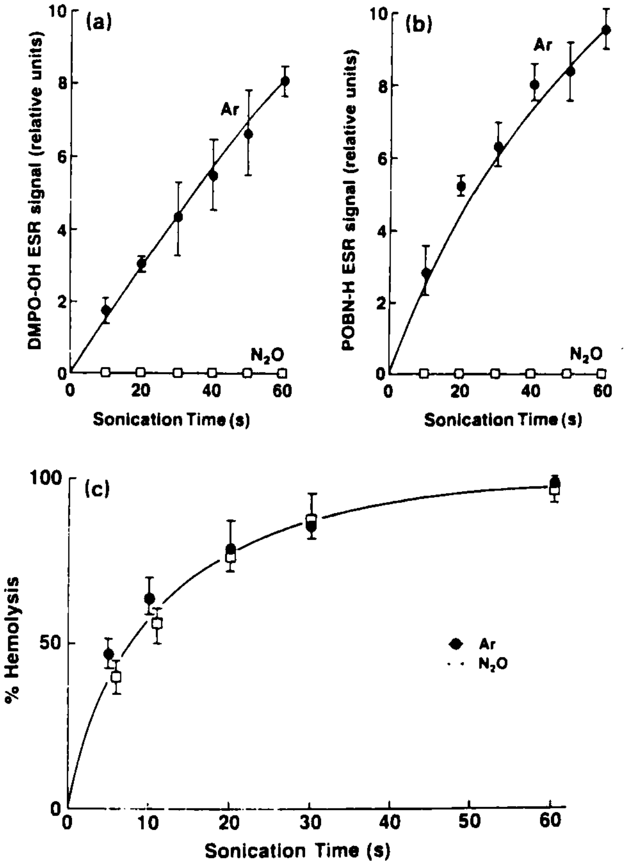

FIGURE 3(a,b) Effect of Ar and N_2O on the free radical formation induced by 50 kHz ultrasound. (a) OH radical formation measured by the DMPO-OH ESR signal; DMPO, 2 mM. (b) H atom formation measured by the POBN-H ESR signal; POBN, 20 mM. (c) Effect of Ar and N_2O on the lysis of erythrocytes (5% hematocrit) exposed to 50 kHz ultrasound. From ref.[34].

transient cavities proposed by Flynn (personal communication).[33] In summary, the production of free radicals is a temperature-dependent phenomenon while cell lysis by transient cavities is a pressure-dependent process (via radiated shock waves). The model deals with cavities filled with rare gases with inital radii in the range from 0.5 to 50 μm. At or near the inertial threshold for transient cavitation, a pressure-dependent phenomenon such as cell lysis is predicted to be fairly constant in magnitude as the thermal conductivity of the rare gas changes, while a temperature-dependent phenomenon such as free radical production should increase in magnitude as the thermal conductivity of the rare gas decreases.

For the triatomic gas N_2O with $\gamma = 1.30$, the final collapse temperature is below the value for which appreciable thermal dissociation of H_2O into H atoms and OH radicals occurs. Thus, by comparing argon and nitrous oxide saturated aqueous solutions, one can distinguish between cavitation with or without OH and H radical formation. No formation of DMPO-OH and POBN-H was found in N_2O-saturated aqueous solutions in contrast to argon-saturated solutions as shown in Figure3(a). However, Figure 3(b) illustrates that the same degree of lysis of erythrocytes was found with argon- and N_2O- saturated solutions (at 5% packed cell volume) exposed to 50 kHz ultrasound. The membrane fluidity, permeability. and deformability of the remaining unlysed erythrocytes after sonication in the presence of Ar or N_2O were unchanged and identical to those of the control cells.[34] On the other hand, after gamma irradiation (700 Gy), the lysis behaviour was quite different from that after sonication, and the membrane properties were significantly changed. These results suggest that lysis induced by sonication was due to radiated shock waves arising from transient cavitation, and that the membrane integrity of the remaining erythrocytes after sonication was the same as that of control cells without sonication.[34]

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