Does salting-out effect nucleate nanobubbles in water: Spontaneous nucleation?

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ABSTRACT
The solubility of gases in aqueous salt solution decreases with the salt concentration, often termed the “salting-out effect.” The dissolution of salt in water is followed by dissociation of salt and further solvation of ions with water molecules. The solvation weakens the affinity of gaseous molecules, and thus it releases the excess dissolved gas. Now it is interesting to know that what happens to the excess gas released during salting-out? Since it is imperative to note that the transfer of dissolved gas in the bulk liquid may often occur in the form of nanobubbles. In this work, we have answered this question by investigating the nano-entities nucleation during the salting-out effect. The solubility of gases in aqueous salt solution decreases with the salt concentration, and it is often termed as the “salting-out effects.” The dissolution of salt in water undergoes dissociation of salt and further solvation of ions with water molecules. The solvation weakens the affinity of gaseous molecules, and thus it releases the excess dissolved gas. Now it is interesting to know that what happens to the excess gas released during salting-out? While it is also imperative to note that the gas transfer in the bulk liquid often occurs in the form of bubbles. With this hypothesis, we have experimentally investigated that whether the salting-out effect nucleates nanobubble or not. What is the strong scientific evidence to prove that they are nanobubbles? Does the salting-out parameter affect the number density? The answers to such questions are essential for the fundamental understanding of the origin and driving force for nanobubble generation. We have provided three distinct proofs for the nano-entities to be the nanobubbles, namely, (1) by freezing and thawing experiments, (2) by destroying the nanobubbles under ultrasound field, and (3) we also proposed a novel method for refractive index estimation of nanobubbles to differentiate them from nano drops and nanoparticles. The refractive index (RI) of nanobubbles was estimated to be 1.012 for mono- and di-valent salts and 1.305 for trivalent salt. The value of RI closer to 1 provides strong evidence of gas-filled nanobubbles. Both positive and negative charged nanobubbles nucleate during the salting-out effect depending upon the valency of salt. The nanobubbles during the salting-out effect are stable only for up to three days. This shorter stability could plausibly be due to reduced colloidal stability at a low surface charge.

1. Introduction
Nanobubbles in a bulk liquid have received significant attention from the past decades. They are gas-filled bubbles of mean diameter of ranging from 100 to 200 nm. Bulk nanobubbles possess distinct features such as extraordinary longevity, high surface to volume ratio (≈5), high mass transfer coefficient and Laplace pressure, ability to generate reactive oxygen species. Owing to such peculiar properties, nanobubbles have wide range of applications in the field of wastewater treatment [1,2], medical application [3], food processing [4], environmental sectors [5], bio-engineering sectors [6], flotation [7], therapeutic delivery [8]. Despite such overwhelming applications, the fundamental understanding of nanobubble generation [9] and a unique characterization technique to distinguish nanobubbles from the particle and nanodroplet [10] are still under infancy. Broadly, nanobubble generation methods include acoustic cavitation [11], porous membrane [12], repeated compression and decompression [13,14], electrolysis [15], external electric field [16] and alternating magnetic field [17]. Several characterization techniques have been employed to detect nanobubbles. For instance, optical methods such as dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), flow cytometry, etc., provide a measurement of size and concentration of nanobubbles but do not

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distinguish nanoparticles and nanodroplets from the nanobubbles [15,16,18]. Freezing and thawing, vacuum, and centrifugation are the class of techniques based on physical perturbation [10], phase contrast microscopy techniques are based on the estimation of the refractive index of nanobubbles which may lie between air (RI = 1.00) and water (1.33). On the other hand, the refractive index of solid particles and droplets is supposed to be greater than 1.33 [19]. The off-axis digital holographic microscopy (DHM) enables the estimation of both refractive index and the size of nanobubbles [20].

We are interested to understand what happens to the excess released gas during the salting-out effect. In general, the gas transfer in the liquid often occurs in the form of bubbles. Therefore, it can be speculated that excess dissolved gas may nucleate in the form of nanobubbles. Does the salting-out parameter play a role in nanobubble nucleation? The solubility of gas decreases in a salt solution with reference to pure water, the so-called salting-out effect. The quantitative study on the solubility of a gas in the electrolyte was reported by Setchenov where he proposed an empirical relation between electrolyte concentration and gas solubility as follows [21]:

$$\log \left( \frac{a_o}{\alpha} \right) = KC_o$$  \hspace{1cm} (1)

where, $a_o$, $\alpha$ and $C_o$ are the oxygen solubility in pure water, oxygen solubility in salt solution of the concentration, $C_o$, $K$ is the salting out parameter depends on the gas type, temperature and valency of the salt. For a low electrolyte concentration, Eq. (1) can be modified using ionic strength as follows [22]:

$$\log \left( \frac{a_o}{\alpha} \right) = hI$$  \hspace{1cm} (2)

where $I$ is the ionic concentration as follows

$$I = \left( \frac{1}{2} \right) \sum c_i z_i^2$$  \hspace{1cm} (3)

For a mixed electrolyte system Eq. (2) can be modified as follows [23]:

$$\log \left( \frac{a_o}{\alpha} \right) = \sum H_i I$$  \hspace{1cm} (4)

where $H_i$ is the specific salting-out constant for ions and the gas. Thus, the solubility of the gas in a mixed salt can be predicted by Eq. (4).

It is readily clear from Eq. (4) that the higher the salting-out parameter value lower the gas solubility in a given salt solution. It is also to be noted that the solubility of gas increases with the valency of the salt. In other words, the solubility difference ($a_o - \alpha$) decreases with salt valency. Several interesting questions can be posted here. For instance, does excess dissolved gas nucleate in the form of nanobubbles? What is the evidence that they are nanobubbles? What is bubble number density in mono-, di- and tri-valent salt solutions during the salting-out effect? How stable are these nanobubbles? What is the mean diameter of the nanobubbles in mono-, di- and tri-valent salt solutions? Does bubble number density increase with salt concentration as the solubility difference increases from Eq. (1)? Are these bubbles stable in the acidic medium? Are these bubble charged? This work endeavors to answer these questions throughout this paper.

From a fundamental research viewpoint, Bunkin et al. [19] performed the experiments on aqueous NaCl solution and characterized by phase microscopy, DLS, and polarimetric scatterometry. The refractive index of micron-sized bubbles was estimated to be 1.26. The mechanism of charging of the bubble essentially depends on the ion present in the solution. Bunkin et al. [24] reported that anions play a major role than the cations in the stabilization of bubbles. Due to the surface charge on the nanobubble, the colloidal stability has been investigated by DLVO theory, and the energy barrier was found to vary from 20 k_BT to 60 k_BT [18] depending upon the pH of the solution. Such a positive energy barrier warrants the colloidal system to be stable. More recently, the ion adsorption at the interface is explained by solving the modified Poisson-Boltzmann equation. The surface charge calculation by modified Langmuir adsorption expressions has been seen in good agreement with the experimental results over a wide range of pH [25]. The accumulation of surface charge density stabilizes the bubbles as the electrostatic pressure ($P_e \approx k_T$) diverge must faster than the Laplace pressure ($P_l \approx \frac{2}{R}$) [18,26–28]. Interestingly, the nanobubbles are observed to be stable against temperature. The mean diameter of the nanobubble decreases with temperature but restores its original size upon cooling the nanobubble sample. Evidently, the temperature may enhance self ionization of water and mobility of ions, and nanobubble stabilization may occur by charge redistribution [29].

The applicability of the Young–Laplace equation and Henry’s law for nanobubbles are recently validated with experimental results [30]. The validity of the Young–Laplace equation facilitates the estimation of the internal gas pressure of nanobubbles. Shi et al. [31] compared the internal pressure by the two methods, namely, by contact mechanics approach and by using the Young–Laplace equation. The contact mechanics model utilizes the force between the bubble and probing tip measured by atomic force microscopy. Both models reported consistent internal pressure in the range of (8–16 bar) for 200 to 400 nm nanobubbles. One of the distinctive properties of nanobubbles is the formation of OH- radicals. The recent measurement based on electron paramagnetic resonance spectrum suggests the existence of OH- radicals [32]. Perhaps this is one of the promising properties of nanobubbles, as free radicals are responsible for the degradation of many organic matters. More recently, nanobubbles in salt solution were generated by hydrodynamic cavitation (external energy devices) method by Hewage et al. [33] and concluded that as the valency of the salt increases the surface charge on the bubble neutralizes and it further leads to the charge reversal. The co-ions and counter-ions have been shown to have negligible effect on the surface potential or the surface charge.

In a nutshell, it is reasonably clear that most of the literature is devoted on understanding the properties and colloidal stability of the nanobubbles. There are no prior studies investigating the origin and generation mechanism of the nanobubbles during the salting-out effect. We have presented a novel method to estimate the refractive index of nanobubbles based on the scattering power measurement. The effect of the valency of salt on nanobubble density, size, and charge at the surface of the nanobubbles has been studied extensively. The temporal stability, refractive index, and the effect of storage containers have been reported. The evidence for nanobubbles is provided by estimating the refractive index of nanobubbles using Mie scattering theory based on the experimental data on scattering intensity. Cryo-TEM imaging of nanobubbles further supported the characterization of nanobubbles. The different storage conditions also play an essential role on the long-term stability of nanobubbles. Extensive measurements of bubble size distribution are reported to understand the effect of storage conditions. Nanobubble stability in alkaline and acidic medium is also investigated.

2. Experimental methods

2.1. Materials

A double distilled water having the electrical conductivity of 1.695 $\mu$Scm$^{-1}$ and pH of 7.1 at a temperature of 25 °C has been used in all the experiments. Aluminium chloride (AlCl$_3$, 99.9%), sodium sulphate (Na$_2$SO$_4$), sodium chloride (NaCl, 99.5%) and calcium chloride (CaCl$_2$, 99%) were purchased from Merck Chemicals. A double distilled water is used to prepare all the stock solutions required for the experiments. A magnetic stirrer is used for 45 min to mix the powder chemicals into the water. The water used for the experiments and a stock solution was examined using NanoSight before the experiments. This is to make sure that there is no contamination at nanoscale present a priori. Additional
precautions during the cleaning and handling of the experimental rig have been taken care of. The disposable latex-free syringes, vials, and pipettes were used to avoid any form of contamination.

2.2. Preparation of nanobubble sample

Nanobubble samples were prepared by dissolving salts in pure water at room temperature. A magnetic stirrer is employed for dissolving salt at 1100 RPM for 45 min. The nanobubble formed during salt dissolution was stored at room temperature in 15 mL air-tight vials for further analysis. The procedure adopted here is described by the schematics in Fig. S1 in the Supplementary material. From thermodynamic viewpoint, the change in free energy during the bubble generation is given as follows:

\[ \Delta G = \frac{4}{3} \pi R^3 G_i + 4\pi R^2 \gamma \]  

(5)

where \( R \) is the radius of the bubble, \( G_i \) is the energy associated with the per unit volume of gas and it is a negative number. Therefore, increasing value of \( G_i \) favours the bubble generation. On the other hand, the surface tension, \( \gamma \) of the liquid is always positive and increasing the value of surface tension hinders the bubble generation. Most of the bubble generation techniques such as acoustic cavitation [11], porous membrane [12], repeated compression and decompression [13], electrolysis [15], external electric field [16] and alternating magnetic field [17] supply external physical energy to increase the value of \( G_i \). During salting-out effects, the Gibbs free energy of mixing tends to enhance the value of \( G_i \), and therefore facilitates the nanobubble generation. The nanobubble generation by physical methods exploit the reduction in the pressure which reduces the solubility of the gas and therefore it tends to nucleate nanobubble. In present technique, the reduction in the solubility of the gas is achieved by dissolution of the salt which leads to the supersaturation of dissolved gas and forms nanobubble.

2.3. Refractive index estimation

The refractive index of the nanobubbles has been estimated by measuring (maximum) the scattering power of the nanobubble sample, \( P_s \) (AU) by NanoSight NS300 and Zetasizer nano have been described extensively in Supplementary material. The measured scattering power of a standard polystyrene nanosphere of known size and refractive index is used to calculate the scattering cross-section \( \sigma_m \) (nm²) from the Mie theory [34]. MieConScat calculator v1.1.8 [35] has been used to calculate scattering cross-section of known size and refractive index of the particle. The MieConScat [35] utilizes the particle size, refractive index of both particle and medium, and the wavelength of the light source as the input parameter for the calculation of theoretical scattering cross-section. Mie theory is based on the analytical solution of Maxwell’s equations describing the scattering of electromagnetic waves around the spherical objects. The solutions of Maxwell’s equations are expressed in terms of infinite series of vector spherical harmonics. MieConScat [35] is the software based on Mie scattering theory, and the details of the calculation procedure are described elsewhere [34]. NanoSight NS300 is calibrated by standard polystyrene nanospheres of the known size (four sizes of polystyrene beads, namely 50 nm, 100 nm, 150 nm, and 200 nm, and concentration 10⁸ particle.mL⁻¹). The refractive index of polystyrene nanosphere is 1.633 [36]. The measured \( P_s \) value has been correlated with the corresponding value of \( \sigma_m \) calculated from Mie theory using MieConScat[35]. A scaling factor \( \phi_{true}/P_s \approx 0.0673 \) is obtained by least-square fitting of the data points. This scaling factor is specific to the tracking instrument (i.e., NS300). All the measurements were performed at 22 °C and 0.95 cP.

2.4. Sample preparation for Cryo-TEM imaging

Nanobubbles in salt solution were visualized using an FEI Tecnai G2 12 Twin TEM 120 kV equipped with a low-temperature Gatan unit. The nanobubble samples were prepared for Cryo-TEM analysis by placing 1 μL of nanobubble solution onto the holey carbon-coated gold grid, and it is further vitrified into the liquid ethane by using Vitrobot(FEI Company). Subsequently, the grid containing the vitrified nanobubbles has been placed onto the Cryo holder, and images were taken at an acceleration voltage of 120 kV at temperature −174 °C. Nanobubbles generated during the dissolution of NaCl with 1 mM salt concentration have been characterized by Cryo-TEM as shown in Fig. 1a. Evidently, the spherical nanobubbles are visualized by Cryo-TEM; however, the size of nanobubbles is larger than that of the NTA measurement as shown in 3-D histogram in Fig. 1b.

3. Results and discussion

3.1. Physical perturbation of nanobubbles

The evidence of the gas-filled nano-objects has been a challenge. The sizing by optical techniques relies on the Brownian motion of the nanobubbles; thus, it does not distinguish between bubbles and particles. In this work, we have provided multiple evidence of the nanobubble nucleated during the salting-out effect. The first approach is the so-called physical perturbations to the nanobubble suspension. The disappearance of nanobubbles during freezing and thawing provides an indication of a soft interface. However, this may also lead to formation of aggregates during freezing and thawing. This has been verified by performing freezing and thawing of polystyrene nanospheres in water. Particle counts and mean diameter were observed to be more or less invariant of freezing and thawing. This observation strengthens the less probability of particle aggregation during freezing and thawing. However, it does not rule out the possibility of coalescence of oil nanodroplets if a small fraction of oil entered into the system. Three nanobubble samples, namely 0.1 mM, 10 mM, and 100 mM, were prepared and characterized by NTA. A representative bubble size distribution for 100 mM sample is shown in Fig. 2a. 10 μL of each nanobubble sample were frozen at a temperature of −18 °C for 12 h which is significantly lower than that of the freezing point of the 1 mM salt solution (≈−3.72 °C). Nanobubble samples were further defrosted at room temperature for about 2 h before characterization by NTA. Typical results on the bubble number density are shown in Fig. 2b. At low salt concentration, for instance, at 0.1 mM of the salt, most of the nanobubble disappears; however, the remaining entities after thawing increase with the salt concentration. The corresponding NTA micrograph before freezing and after thawing are shown in Fig. 2c and d. The bubble count per frame reduced from 17 to 3 bubbles per frame that is ~82% decrease in the bubble count. Other concentration of salt also exhibit a similar trends (see Fig. S2 in Supplementary materials).

Other physical perturbation techniques, such as pressurization, vacuum, and centrifugation, have also been employed in the nanobubble sample in the recent past. The size of nanobubble increases, and bubble number density decreases by pressurization of nanobubble sample [37]. Such a compressible behavior of the suspension indicates the gas-filled nano-entities. In contrast, employing a vacuum to the nanobubble sample [38], mixing degassed water with nanobubble sample [39] exhibit disappearance of nanobubbles. Direct irradiation of ultrasound in the nanobubble sample also leads to the disappearance of nanobubbles, as shown in Fig. 3. 20 kHz frequency of ultrasound for 5 min in a continuous mode have been employed in each of the experiments. The amplitude is varied from 25% to 98%, 60 μL of nanobubble sample is exposed to titanium ultrasound probe with varying amplitude. At a low percentage amplitude, the bubble number density was unaffected by the ultrasound, while it reduces ~ 40 –50% at a higher amplitude. It is to be noted that ultrasound also generates nanobubbles in pure water at one
hand but also destroys the nanobubbles already present in the solution. From a theoretical standpoint, the pressure generated by ultrasound, the so-called acoustic pressure wave, gives rise to compression and the rarefaction cycle. During the rarefaction cycle, pressure in the liquid falls below the vapor pressure of the liquid and thus results in the nucleation of micro and nanobubbles. The bubble generation during the rarefaction cycle is only possible if there are gas source present. If the system contains bubbles a priori, then the compression cycle tends to destroy the bubble, and therefore, we observe a significant reduction in the nanobubble population if the irradiate nanobubble solution with ultrasound. The surface charge on the nanobubble significantly increases with ultrasound, but the mean diameter was found to be unaffected (see Fig. S3 in Supplementary material). It is readily apparent that nano drops may also behave similar to the nanobubbles by the physical perturbation mentioned above. Therefore, the evidence for nanobubbles is further strengthened by estimating the refractive index of the nano-entities in various salt solutions, as discussed in the next section.

3.2. Refractive index of nanobubbles

Refractive index is related to the chemical composition of the suspended material, and it is often used to differentiate different constituents in the medium. For instance, extracellular vesicles in biological samples (RI < 1.42) can be distinguished from the lipoproteins (RI > 1.42) of similar size based on refractive index [41]. The refractive index of oil drops in water [42] and nanoparticles [43] were reported to be higher than 1.33. In this work, we have estimated the refractive index of nano-entities formed during the salting-out effect for mono-, di- and tri-valent salts as shown in Fig. 4(a) and (b). As noted earlier, the scattering power of known size and refractive index of polystyrene nanospheres have been used to calibrate the NTA. The instrument scaling factor is used to compare the measured scattering cross-section and theoretical

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Fig. 1. (a) Cryo-TEM micrograph of nanobubbles in 1 mM of NaCl solution (b) 3D histogram of bubble count, size and light scattering intensity in nanobubble sample of 1 mM NaCl.

Fig. 2. Freezing and thawing experiments on nanobubble sample generated by dissolution of NaCl (a) bubble size distribution before freezing and after thawing (b) bubble number density before freezing and after thawing (c) NTA micrograph before freezing (d) NTA micrograph after thawing.
from Mie theory calculations to estimate the refractive index of the nanobubble suspension. Fig. 4(a) demonstrates the overlap of measured scattering cross-section and theoretical value from the Mie theory. For nanobubbles in mono and divalent salts, the refractive index value was estimated to be in the range of 1.011–1.023, whilst the refractive index of the nanobubbles in tri-valent salt was calculated to be \( \sim 1.305 \). Such a higher value of the refractive index of nanobubbles in trivalent salt is plausibly due to the absorption of \( \text{Al}^{3+} \) around the nanobubble interface leading to a slight increase in the opacity. Interestingly, it is to be noted that the refractive index of nanobubbles in all the mono-, di-, and tri-valent salt is less than that of 1.33. Evidently, this is within the range of gas bubbles. The present findings are observed to be in line with the reported results of air nanobubbles [20]. The refractive index estimation using digital holographic microscopy is based on the calculation of the phase shift of the illuminated light. Particles exhibit a positive phase shift, while bubbles possess a negative phase shift when the light is illuminated. The integrated phase shift versus mean diameter enables the estimation of the refractive index of bubbles and particles.

The ultrasound field has also been used as a part of the physical perturbation technique for the evidence of nanobubbles. The direct irradiation of ultrasound on nanobubble sample destroys the nanobubbles [5]. We have estimated the refractive index of the nanobubbles generated in 0.1 and 1 mM of NaCl salt by varying the ultrasound amplitude as shown in Fig. 4(b). It is to be noted that the refractive index is estimated after the ultrasound as it requires to reduce the temperature for NTA analysis. For ultrasound experiments, 50 mL of nanobubble suspension were placed in a beaker. The ultrasound probe was dipped in the solution and irradiated for 5 min in a continuous mode. The estimated values of the refractive index were shown to be invariant with the amplitude used. This effect probably suggests that ultrasound is not introducing any contamination even at a higher amplitude. In a nutshell, we have provided evidence for nanobubble generation during salting-out effects. The freezing and thawing experiment concludes that the suspension has soft interfaces. Furthermore, refractive index estimation provides another exciting piece of evidence for nanobubbles. Refractive index increases slightly in the case of tri-valent salt, and probably it is due to the absorption of \( \text{Al}^{3+} \) at the interface.

3.3. Influence of salt valency

Nanobubble generation during the salting-out effect is characterized by NTA in terms of bubble size distribution, bubble number density, and mean size. Surface charge is measured by zeta potential. The concentration of various salts has been varied up to 5 mM to understand the effect of salt concentration on nanobubble generation. Both Bubble number density and size of the nanobubbles increase with the concentration of the salt, and it is observed to be irrespective of the valency of the salt as shown in Figs. 5 and 6. However, the surface charge was observed to be more or less invariant with the salt concentration. Nanobubbles in NaCl, CaCl₂, and Na₂SO₄ were measured to be negatively charged while the nanobubbles in AlCl₃ possess a positive charge. This observation is found to be inline with the nanobubbles in salt solution generated by hydrodynamic cavitation route [33]. The comparison of bubble number density for various salts is performed after concentrating the nanobubble sample using a Rotary evaporator. 250 mL of nanobubble suspension were reduced to 20 mL by evaporating the solvent. A typical comparison of bubble number density, bubble size distribution, and zeta potential of different salts is shown in Fig. 6. Evidently, bubble number density in mono-valent salt is observed significantly higher than in the di- and tri-valent salt. The mean diameter of the nanobubble was observed to be slightly increased for di-valent salts (CaCl₂ and Na₂SO₄) in comparison with NaCl, as shown in Fig. 6. However, further the mean diameter of nanobubble decreases in the tri-valent salt (AlCl₃). Interestingly, the surface charge on the nanobubbles in the AlCl₃ solution is measured to be positive. The zeta potential of nanobubbles in deionized water provides the speculation of the negative charged air-water interface [18]. Such charged nano-entities form an electric double layer around the objects. The strong positive ions such as \( \text{Al}^{3+} \) may absorb in the interface, and therefore nanobubbles may exhibit a positive value of zeta potential.

The salting-out parameter denotes the slope of the solubility plot. A higher value of the salting-out parameter results in the low solubility of the gas. Table 1 shows the typical value of the salting-out parameters for the salts used in this work. Clearly, the value of the salting-out parameter is significantly higher in the case of NaCl than the other di- and trivalent salts. For a fixed value of the salting-out parameter (K), the

**Fig. 3.** Effect of ultrasound amplitude on bubble number density of nanobubble sample during salting-out effect in NaCl solution. The frequency of the ultrasound used here is 20 kHz for 5 min in a continuous mode. The amplitude is varied from 25% to 98%.

**Fig. 4.** (a) Representative scattering cross-section for nanobubbles in NaCl, CaCl₂, Na₂SO₄, and AlCl₃ solution versus mean bubble diameter (b) Refractive index of nanobubble sample of 0.001 mM and 1 mM of NaCl.
ratio of solubility expressed in terms of Bunsen coefficient varies proportionally with the concentration of the salt as shown in Eq. (1). For a fixed temperature and pressure, the solubility of the gas in pure water is constant; therefore, the solubility of the gas in the salt solution decreases with increased concentration. This correlates reasonably well with the increasing bubble number density with the salt concentration, as shown in Fig. 6. In other words, the solubility difference ($\alpha_o - \alpha$) increases with salt concentration that is the available gas for nanobubble nucleation. The salting-out parameter also explains the higher bubble number density in mono-valent salt compared to di- and tri-valent salt. The

Fig. 5. Nanobubbles in NaCl solution of varying concentration (a) Zeta potential (b) Mean bubble diameter (c) Bubble number density. The pure water is analysed using NTA before adding the salt, no nano-entities were observed.

Fig. 6. Nanobubbles in Na$_2$SO$_4$ solution of varying concentration (a) Zeta potential (b) Mean bubble diameter (c) Bubble number density. The pure water is analysed using NTA before adding the salt, no nano-entities were observed.
salting-out parameter for monovalent salt is significantly higher than that of the di- and tri-valent as shown in Table 1. Clearly, the slope of the lines on \( \log(C_0) \) versus \( C_0 \) is expected to be much lower for di- and tri-valent salt, and therefore, for a given salt concentration, the solubility of the gas in di- and tri-valent salt is expected to be higher with reference to mono-valent salt. Thus, the solubility difference \( (\alpha_o - \alpha) \) reduces for di- and tri-valent salts, and this is probably reflecting in terms of less bubble number density in di- and tri-valent salt shown in Fig. 7. In addition, the other plausible reason for decreased bubble number density could be the dissolution of smaller nanobubbles in the presence of stronger cations. Also, the electric double layer deteriorates faster in di- and tri-valent salts. The bubble size distribution of nanobubbles in different salts are shown in Fig. S4 in Supplementary material. In summary, higher values of the salting-out parameter exhibit a positive correlation with the bubble number density. Similarly, increasing salt concentration also enhances the bubble number density due to the higher solubility difference with the salt concentration. The decrease in bubble number density in di- and tri-valent salt may also be because of the faster de-stabilization of the electric double layer.

### 3.4. Temporal stability of nanobubbles in salt solution

Nanobubbles in pure water have been seen to be extraordinarily stable for days and months [31,44,45]. Bubble number density decreases monotonically while mean diameter increases 30–40% over the period of time. Since nanobubbles in pure water are negatively charged, bubble coalescence and dissolution can possibly be ruled out. Therefore, the disappearance of the nanobubbles is expected from the air–water interface at the top and by collision with the storage container wall. It is also to be noted that the mean square displacement of the smaller bubble is higher with respect to the larger bubble from Einstein-Stokes equation. In other words, the probability of collision for smaller nanobubbles is more than the larger bubbles. Thus, the smaller bubble may disappear faster than the larger bubble. This might be the possible justification for the increasing mean bubble diameter over a period of time as a smaller bubble is expected to disappear earlier. In this work, we have examined the so-called long-term stability of nanobubbles in NaCl solution. The bubble number density was significantly higher in NaCl solution, and therefore the sample is chosen for long-term stability. The nanobubbles in salt solution are seen to disappear much faster than in pure water, as shown in Fig. 8. Nanobubbles are observed to be stable for no more than three days. The mean diameter of the nanobubble increases by \( \sim 37\% \) in three days. This is in line with the nanobubbles in a pure water system [45,31]. The faster decay of the bubble number distribution in salt solution may be attributed to the presence of salt that leads to an electric double layer screening around nanobubbles. Weak surface charge on the nanobubbles give rise to lower colloidal stability of the system, and therefore, nanobubble disappears must faster in a salt solution.

As noted earlier, the disappearance of nanobubbles is because of the collision with the storage container. Perhaps perturbing the type of container wall may provide some insight into this hypothesis. We have performed the experiments on the effect of a storage container on the stability of nanobubbles as shown in Fig. 8. Two 15 mL vials, namely, the glass and polypropylene material, have been used. The volume of stored nanobubble samples is kept to be equal. Evidently, the bubble number density decays faster in polypropylene vials than that in glass vials. The plausible reason could be the absorption of nanobubble inside the polypropylene vial. Indeed, it is difficult to claim the exact mechanism of nanobubble absorption in polypropylene containers, but the hypothesis on the nanobubble disappearance by striking the container wall seems possible. In addition, temperature and pressure conditions may also influence the stability of the nanobubbles. Brownian motion of nanobubbles is expected to be higher at high temperatures; therefore, the probability of collision with the container wall is expected to be enhanced. This, in turn, leads to a high rate of disappearance of nanobubbles at a higher temperature. This hypothesis is observed in line with

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**Table 1**

| Salt   | \( K (\text{dm}^3/\text{mol}) \) | Molar concentration range (M) |
|--------|---------------------------------|-------------------------------|
| NaCl   | 1.145                           | 0–5                           |
| CaCl\(_2\) | 0.226                          | 0–4.5                         |
| Na\(_2\)SO\(_4\) | 0.376                          | 0–1.2                         |
| AlCl\(_3\) | 0.274                          | 0–1.7                         |

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**Fig. 7.** Nanobubbles in salt solution of different valency (a) Zeta potential (b) Mean bubble diameter (c) Bubble number density.
In a nutshell, nanobubbles during salting-out effects are observed to be stable for three days, much less than that of the pure water system. The plausible reason could be the weak colloidal stability in the presence of salt. Nanobubbles in polypropylene container decays faster than that in glass vials. Nanobubble may disappear by striking the storage container.

3.5. Nanobubbles in acidic and alkaline medium

Nanobubbles in pure water (at neutral pH) are extraordinarily stable.
It is also known that nanobubbles are more stable in alkaline medium [18,15]. Bubble number density drops in the acidic medium, whereas mean diameter increases. Surface charge approaches toward an isoelectric point in the acidic medium. It is also interesting to note that nanobubbles can be further charged negatively by adjusting the pH of the sample to the alkaline limit. This is plausibly due to the absorption of OH− at the air–water interface. This behavior is qualitatively similar to that of any charged nano-suspension. In this work, nanobubbles in NaCl solution are concentrated using a rotary evaporator for pH adjustment. Hydrochloric acid and sodium hydroxide are used to adjust the pH of the sample. Nanobubbles in the salt solution behave qualitatively similar to that in the pure water, as shown in Fig. 9. Bubble number density decreased by ∼ 30–50% from pH = 10 to pH = 3.5. Similarly, the mean bubble diameter of the nanobubble increases by 30–40%. On the other hand, zeta potential approaches toward ∼ −32 mV at pH = 10. Bubble size distribution is reported in Fig. S5 in Supplementary material. A higher value of zeta potential denotes the strong electrostatic interaction and thus the stronger colloidal stability. The higher mean bubble diameter at lower pH may be due to the screening of the electric double layer. The addition of H+ ion is expected to neutralize the air–water interface. The smaller size fraction of nanobubbles may be dissolved or shrink much faster than that of the larger bubble due to the screening of the electric double layer. Therefore, the measured diameter of nanobubbles at low pH exhibits a relatively larger mean. In summary, nanobubbles in salt solution behave similar to that of in pure water. Surface charge decreases in the acidic medium due to the screen of the electric double layer by H+ ion. On the other hand, nanobubbles are expected to be more stable in the alkaline medium due to the strong electrostatic interaction that gives rise to the higher total interaction potential.

4. Conclusions

Finally, we conclude that the excess dissolved gas indeed nucleates in the forms of nanobubbles during the salting-out effect based on the refractive index calculation. Further evidence of nanobubbles was supported by the freezing and thawing process. A novel method for the estimation of the refractive index of nanobubble is proposed based on Mie theory calculations. The measured scattering power by NTA is utilized to calibrate the NTA using standard latex nanospheres and to estimate the refractive index of an unknown sample. The refractive index of nanobubble samples was calculated to be 1.012 for mono and di-valent salt while 1.302 for the tri-valent salt. The absorption of Al3+ ion in the electric double layer around the nanobubble is the expected reason for the increase in the refractive index of tri-valent salt. The bubble number density was observed to increase with the salt concentration, which is explained by the Setchenov solubility relation for electrolytes. The solubility difference increases with the salt concentration, and therefore, bubble number density exhibits a positive correlation with the salt concentration. We also conclude that bubble number density depletes with the salt valency. This is explained by using a salting-out parameter; the higher the value of the salting-out parameter lower the solubility of the gas in the solution. Therefore, the solubility difference yields a positive dependence on the salting-out parameter. The reported values of the salting-out parameter [22] corroborate the observed experimental trends.

We also conclude that nanobubbles during salting-out effects are only stable for up to three days, which is much shorter than nanobubbles in pure water. NaCl, CaCl2 and Na2SO4 nucleate negatively charged nanobubbles whereas AlCl3 salt generates a positive charge nanobubble. We also hypothesize that the nanobubble disappears by the collision of nanobubbles with the container wall. Polypropylene and glass containers were used to store the nanobubble sample in controlled conditions. Nanobubble decays relatively faster than that in glass vials. This confirms that nanobubble disappears by colliding with the storage container. Nanobubbles in salt solution respond similar to pure water to the pH adjustment. Surface charge decreases in the acidic medium due to the screening of the electric double layer. On the other hand, nanobubbles are more stable in the alkaline medium due to the strong electrostatic interaction that gives rise to a higher total interaction potential. The following points describe the utilizations and implications of present results in a larger picture.

- One of the important conclusion of the present work is that the bubble number density increases with the salting-out parameter. In other words, the solubility difference is the driving force for nanobubble generation. This information can be utilized to maximize the bubble number density in the commercial nanobubble generator to enhance performance.
- Stable nanobubbles in salt solution have been demonstrated in this work; however, the stability is much shorter than the nanobubbles in pure water. Water treatment in coastal areas has always been a challenging problem. Since nanobubbles are stable in saltwater and thus the nanobubble generator can be used for water treatment in coastal regions.
- We have proposed a novel technique to estimate the refractive index of nanobubbles based on the scattering power measurement. This technique provides strong scientific evidence of nanobubbles. This technique can be used for the validation and testing of a commercial nanobubble generator.

CRediT authorship contribution statement

Kalyani Agarwal: Conceptualization, Methodology, Writing – original draft. Mohit Tivedi: Data curation, Writing – original draft, Supervision. Neelkanth Nirmalkar: Writing – review & editing, Validation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, athttps://doi.org/10.1016/j.ulsotchn.2021.105860.

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