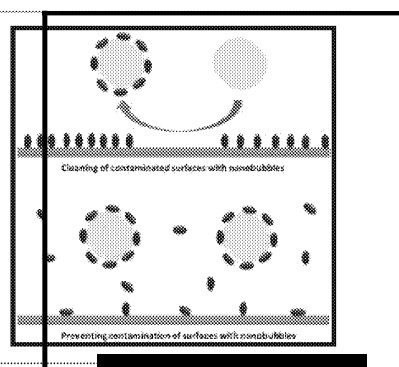


Cleaning with Bulk Nanobubbles

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Supporting Information

ABSTRACT: The electrolysis of aqueous solutions produces solutions that are supersaturated in oxygen and hydrogen gas. This results in the formation of gas bubbles, including nanobubbles ~100 nm in size that are stable for ~24 h. These aqueous solutions containing bubbles have been evaluated for cleaning efficacy in the removal of model contaminants bovine serum albumin and lysozyme from surfaces and in the prevention of the fouling of surfaces by these same proteins. Hydrophilic and hydrophobic surfaces were investigated. It is shown that nanobubbles can prevent the fouling of surfaces and that they can also clean already fouled surfaces. It is also argued that in practical applications where cleaning is carried out rapidly using a high degree of mechanical agitation the role of cleaning agents is not primarily in assisting the removal of soil but in suspending the soil that is removed by mechanical action and preventing it from redepositing onto surfaces. This may also be the primary mode of action of nanobubbles during cleaning.



INTRODUCTION

Nanobubbles or fine bubbles are small gaseous entities that are found on surfaces and in the bulk when solutions are supersaturated with gas.^{1,2} They were first proposed to explain unusual long-range attractive forces observed between hydrophobic surfaces³ and later imaged on surfaces using atomic force microscopy.^{4,5} They are of particular interest as the observed stability of nanobubbles is inconsistent with current theories of bubble dissolution.^{6,7} Moreover, surface nanobubbles exhibit exceptionally high contact angles that cannot currently be explained.⁸ Despite the theoretical challenges in understanding nanobubbles, they are already finding applications in a broad range of areas including medicine,^{9–11} crop production,¹² controlling boundary slip,¹³ and bioremediation.¹⁴ The possible advantages of using nanobubbles in a broad range of applications include the ease with which they can be produced, the low cost of materials, and the potential to easily remove them from the system once they have performed their function. Additionally, they may provide solutions to industrial challenges with low environmental impact. It is for this reason that nanobubbles are seen as promising agents for cleaning.

Cleaning in a scientific sense is often associated with detergency, but common experience tells us that cleaning does not require the presence of a surfactant or detergent if sufficient mechanical means is employed. Our prior work, as well as the work of others, describes the prevention of both fouling and cleaning using surface nanobubbles.^{15–17} The role of nanobubbles in preventing fouling is to provide a mechanical barrier to the adsorption of material on the surface. Thus, the regions

of the surface that are decorated with nanobubbles mask the surface from exposure to contaminants. When employed in this manner, the regions of the surface not covered by nanobubbles will still be fouled, so this is not a preferred mode of operation. However, it was shown that the production of nanobubbles on a surface that is already contaminated results in surface cleaning that can result in nearly all contamination being removed.^{16,17} Nanobubbles in these studies were prepared by electrolyzing water, with the resultant supersaturation of dissolved gas leading to the formation of surface nanobubbles. As the nanobubbles expanded, the advancing three-phase line, which marks the boundary between the nanobubble and the liquid on the surface, removed the contaminant from the surface. It was found that cleaning was more effective with nanobubbles than with sodium dodecylsulfate (SDS) surfactant that is commonly used in detergents and that cleaning was further enhanced when surfactant was also used.^{16,17} Surfactants clean by reducing the adhesive forces between the contaminant and the surface, whereas surface nanobubbles clean when the advancing three-phase line transfers contaminants from the substrate to the gas/solution interface. The high energy of the interface provides for powerful cleaning action and reflects the comparative strength of capillary forces compared to surface

Special Issue: Nanobubbles

Received: March 15, 2016

Revised: April 22, 2016

forces. Thus, cleaning with nanobubbles also involves a mechanical component, in addition to surface energetics. A particular advantage of using nanobubbles for cleaning is that there is no need to rinse surfactant residue from the surface after cleaning has been effected. Thus, nanobubbles have been proposed for use in specialist applications such as cleaning semiconductors.¹⁸ Furthermore, cleaning with nanobubbles has the potential advantages of reducing the considerable environmental impact of surfactant use, lowering the use of nonrenewable resources, and lessening emissions into the environment.

Electrolysis has also been reported to produce nanobubbles in the bulk.^{19–25} Electrolysis results in the supersaturation of oxygen and hydrogen in the anodic and cathodic streams of the solution, respectively, causing bubbles to be nucleated. Larger bubbles quickly rise to the surface and burst, but particle sizing techniques show persistent nanoparticles of around 100 nm following electrolysis that are not present in control solutions. A number of authors have concluded that the nanoparticles produced by electrolysis are gaseous, as described below. This is consistent with the range of methods used for their production, which all involve supersaturation and the absence of nanoparticles in the control solutions that have been investigated.

Kikuchi et al.²³ electrolyzed water to generate solutions supersaturated with oxygen. The formation of nanoparticles in these solutions was detected by light scattering, and their concentration was analyzed by measuring the oxygen content. The dissolved oxygen level was analyzed with a dissolved oxygen (DO) meter and the Winkler titration method.²⁶ It was shown that these methods access only the dissolved oxygen, not that present within bubbles, but by the addition of sulfuric acid, the stability of the nanobubbles was disrupted and a subsequent Winkler titration revealed an increase in oxygen concentration. The difference in oxygen concentration was used to determine the concentration of bulk nanobubbles of oxygen produced during electrolysis. Similarly, in previous work Kikuchi et al.¹⁹ investigated solutions supersaturated in hydrogen gas. By comparing the results from a dissolved hydrogen meter and chemical analysis, they found that the chemical analysis revealed a higher concentration of hydrogen gas and attributed the difference to the existence of hydrogen nanobubbles. Note that the chemical analysis required acidification. This is thought to remove the charge on the nanobubbles and destabilize them.

The production of bulk nanobubbles by mechanical methods of supersaturation has also been reported. Ohgaki et al.²⁷ reported that solutions of bulk nanobubbles consisting of nitrogen, methane, and argon with an average radius of 50 nm were stable for 2 weeks. They were produced by mechanical gas injection, which leads to a supersaturated solution. A scanning electron micrograph of replica films of rapidly frozen and fractured solutions showed a 50-nm-radius cavity. Ushikubo et al.²⁸ reported the production of both oxygen and air-filled nanobubbles by mechanical methods. The size of the nanobubbles was characterized by dynamic light scattering. Additionally, it was claimed that nanobubbles caused an increase in the T1 relaxation time of the solutions as measured by NMR. Kobayashi et al.²⁹ have used an Archimedes microelectromechanical sensor for the detection of nanobubbles. This technique is sensitive to the density of particles; therefore, particles that are positively buoyant are readily distinguished from negatively buoyant particles. Furthermore, the size of the particle is measured independently of the density. Using this technique, nanobubble solutions showed

positively buoyant particles of the same size (~100–250 nm) as particles detected by dynamic light scattering, providing strong evidence for the existence of bulk nanobubbles. When a modulation interference microscope is used, particles can be distinguished on the basis of the refractive index and thereby bubbles (refractive index = 1) can be distinguished from other particles. Bunkin et al. have identified stable nanobubbles in the size range of 250–750 nm using this technique.³⁰

Temperature changes have also been used to produce bulk nanobubbles 290 nm in diameter.³¹ Increasing the temperature reduces the solubility of dissolved gases, resulting in supersaturation. These experiments were performed with the temperature change being induced in a cell mounted in a light-scattering instrument. The conclusion is that particles produced upon increasing the temperature are nanobubbles as the solubility of most materials other than gas will increase with increasing temperature.

Here we test the hypothesis that bulk nanobubbles produced through the electrolysis of aqueous electrolyte solutions enhance the cleaning capacity of the solution. Electrolysis results in the production of hydrogen and oxygen gas as well as a range of other chemical reactions. To control for the effect of chemical changes as opposed to physical changes in the solutions, we compare solutions that have been filtered to remove nanobubbles with those containing nanobubbles in order to determine the effectiveness of the nanobubbles as cleaning agents. Additionally, we use untreated solutions as control samples.

■ MATERIALS AND METHODS

Sodium chloride solutions of concentration 2.1×10^{-3} M were prepared with water that had been purified using either a Milli-Q gradient or an ELGA purelab Chorus 3 system, resulting in a solution with conductivity of $\sim 250 \mu\text{S}/\text{cm}$. Nanobubbles were produced by electrolysis using an ec-H₂O Nanoclean cell that employs five electrode plates of platinum with a plate spacing of 2.79 mm. The device and control electronics is a proprietary design of Tennant Company. Aqueous solution was treated electrochemically using a cell voltage of ~ 24 V at a flow rate of 7.5 mL s^{-1} . This results in the fluid transiting the plates in approximately 13 s. The electrolysis reaction produces oxygen and hydrogen gas in a molar ratio of 1:2 according to the reaction equations shown in Figure 1. Immediately after treatment, numerous visible bubbles are apparent in the solution. Because of buoyancy, the larger bubbles rise to the surface and burst, hence the appearance of the solution changes significantly during the first few minutes after production. Filtering was conducted on selected samples using syringe filters of either a 20 nm (0.02 μm Whatman Anotop 10 inorganic with an Al₂O₃ membrane) or 450 nm (0.45 μm Millipore Millex-LCR Hydrophilic PTFE membrane) pore size.

For cleaning studies, we developed a protocol that could be used to determine if any cleaning observed was due to electrolysis and furthermore could determine the size of the species responsible for such action. This was specifically developed to enable any cleaning due to chemical changes that occur during electrolysis to be separated from cleaning associated with the nanoparticles produced during electrolysis. Note that this is necessary because other reactions will occur in the presence of chloride ions, including the formation of hypochlorous acid. In addition the effluent from the cathode and anode locally will be alkaline and acidic, respectively. It is known that alkalinity in particular can affect cleaning performance. However, the streams are mixed, and the change in pH of the solution upon electrolysis is minimal by the time the solutions are used. Five solutions were studied as depicted in Table 1.

A NanoSight (NS300, Nanosight, software Nanosight V3.1) instrument for nanoparticle tracking analysis using a blue laser light source (70 mW, $\lambda = 405 \text{ nm}$) at 25°C was used to size the

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DOI: 10.1021/acs.langmuir.6b01004
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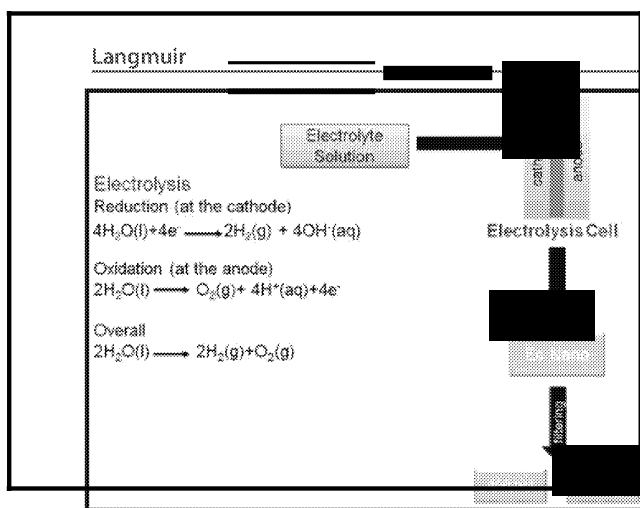


Figure 1. Schematic showing the production of samples for investigation. The electrolysis of 2.1 mM NaCl solutions is used to produce a solution supersaturated with oxygen and hydrogen gases (ec-H₂O), which is used as a test solution. Filtration through a 450 nm filter excludes all particles and bubbles larger than 450 nm and produces a second test solution (ec-H₂O sub 450 nm). Filtration through a 20 nm filter removes the nanoparticles produced during electrolysis and provides a control solution in which the chemical effects of electrolysis are maintained (ec-H₂O sub 20 nm). The untreated electrolyte sample, filtered or unfiltered, was also used as a control solution.

Table 1. Nomenclature for the Solutions Employed to Investigate Cleaning

designation	description
NaCl(aq)	purified water with 2.1 mM NaCl added
NaCl(aq) sub 450 nm	purified water with 2.1 mM NaCl added that has been filtered through a 450 nm filter
ec-H ₂ O	purified water with 2.1 mM NaCl that has been electrolyzed
ec-H ₂ O sub 450 nm	purified water with 2.1 mM NaCl that has been electrolyzed and then filtered through a 450 nm filter
ec-H ₂ O sub 20 nm	purified water with 2.1 mM NaCl that has been electrolyzed and then filtered through a 20 nm filter

nanoparticles and determine the concentration. Each measurement comprised an analysis of five movies, each 60 s long, captured at 25 frames/s. The camera level was set to 14, the threshold to 3, the gain to 366, and the shutter to 31.48 ms, and analysis was conducted using a solution viscosity of 0.888 cP. The Nanosight determines the size of individual particles from their diffusion under Brownian motion using nanoparticle tracking. The concentration is directly determined by counting the number of particles observed in a known volume. The tracking of numerous particles enables the size distribution of particles to be determined. Samples of ec-H₂O were filtered through a 450 nm filter before introduction into the NanoSight measurement cell, to ensure that larger bubbles did not interfere with the measurements. A Zetasizer Nano ZS (Malvern) was also used to measure the size of nanoparticles by dynamic light scattering as well as the zeta potential by laser Doppler microelectrophoresis in an ac electric field.

Proteins bovine serum albumin (BSA), sourced from Hualyuan Biotechnology Co., and lysozyme, sourced from Bio Basic Int., were used as model contaminants. The amount of material was measured using spectroscopic ellipsometry (M2000 V, J. A. Woollam, U.S.A.) at two incident angles of 65 and 75° in air and at an incident angle of 75° in aqueous solutions after the adsorption of contaminants. The refractive index of the contaminant layer was estimated to be 1.45 to evaluate the layer thickness. The contact angle measurements were performed using a KSV (Helsinki, Finland) CAM 200 contact angle goniometer. Both hydrophilic and hydrophobic surfaces were prepared

for cleaning analysis. Silicon wafers were rinsed with water and ethanol and dried with a flow of nitrogen and then cleaned by water plasma treatment at a power of 18 W for 15 min. After these treatments, the silicon wafers were used as hydrophilic surfaces (contact angle ~0°). Hydrophobic surfaces with a water contact angle of ~107° were prepared by modifying the silicon wafers with a hydrophobic monolayer. Specifically, after treatment by water plasma, the activated substrates were modified via vapor deposition of dodecyltrimethoxysilane at 60 °C for 6 h and then rinsed with ethanol and water and dried with a flow of nitrogen to form the hydrophobic monolayer.

RESULTS AND DISCUSSION

Characterization of the Electrolyzed Solutions. We have not directly demonstrated that the nanoparticles produced by electrolysis are gaseous, but the balance of the evidence is that they are indeed nanobubbles. Consistent with previous reports of nanoparticle production from solutions supersaturated by electrolysis, we will henceforth refer to them as nanobubbles.^{19–25} The size and concentration of nanobubbles produced by electrolysis were determined using the Nanosight instrument. Samples of ec-H₂O NaCl were filtered through a 450 nm filter before introduction into the NanoSight cell. The NanoSight revealed a population of nanobubbles of concentration $(750 \pm 50) \times 10^6$ particles/mL (Figure 2). The mean

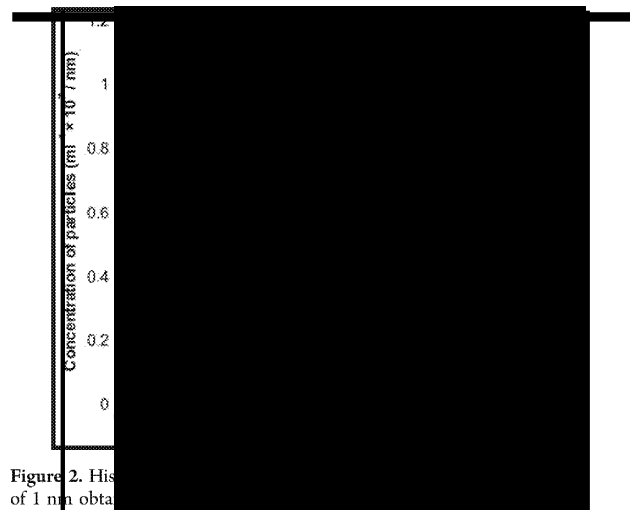


Figure 2. Histogram of the concentration of particles of 1 nm obtained from the NanoSight instrument for NaCl(aq) sub 450 nm, H₂O sub 450 nm, and ec-H₂O sub 20 nm. The total concentration of ec-H₂O sub 450 nm was $(750 \pm 50) \times 10^6$ particles/mL.

size of nanobubbles for the data shown was 112 ± 2 nm with a mode of 93 ± 4 nm. The error bars are from repeated measurements on the same sample. A 10–15% degree of variation is typically seen between samples. As a control, NaCl(aq) was filtered with a 450 nm filter before measurement, revealing a concentration of $(28 \pm 2) \times 10^6$ particles/mL. The only difference was that this sample was not passed through the ec-H₂O electrolysis cell. Filtering ec-H₂O through a 20 nm filter removed the nanobubbles, resulting in a concentration of nanoparticles of $(3 \pm 1) \times 10^6$ particles/mL, which is near the lower limit of the range of the apparatus. Similarly, filtering the laboratory-grade water through a 450 nm filter showed a concentration of nanoparticles of $(3 \pm 1) \times 10^6$ particles/mL. It is also important to ascertain the lifetime or the stability of the nanobubbles that are produced by electrolysis. The stability

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DOI: 10.1021/acs.langmuir.6b01004
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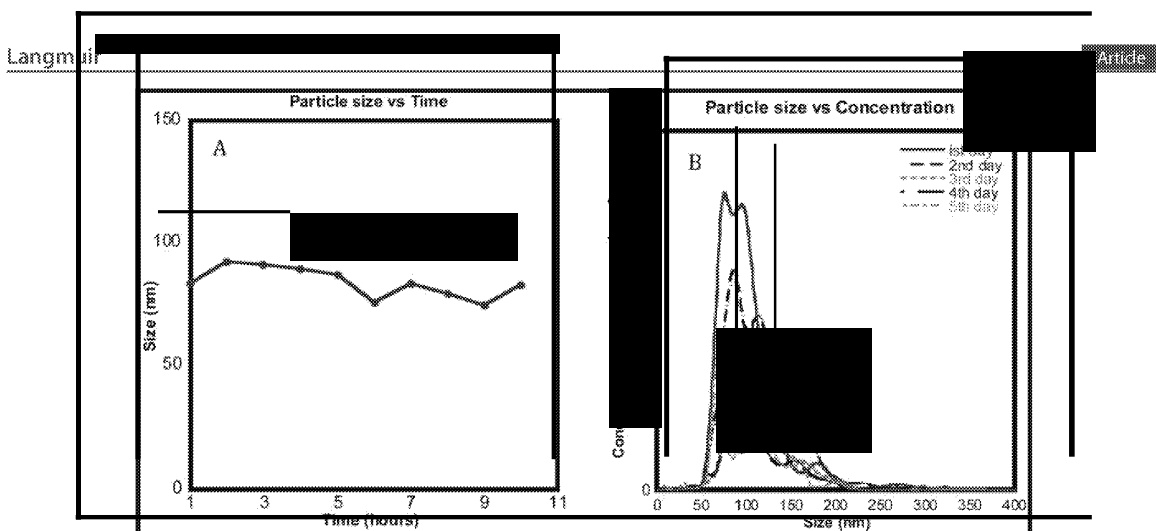


Figure 3. (A) Mean bubble size distribution for ec-H₂O sub 450 nm measured using light scattering over 10 h. (B) Histogram showing the particle concentration in a bin width of 1 nm measured using the NanoSight on samples of ec-H₂O sub 450 nm over 5 days.

of the solutions was first examined using dynamic light scattering (Zetasizer, Malvern). An ec-H₂O sub 450 nm sample was checked at intervals to ascertain the size of the nanobubbles. The cuvette was left in place throughout the measurement. The particle size was found to be stable for 10 h, as shown in Figure 3a. These experiments were hampered by the formation of bubbles on the surface of the cuvette preventing the size of the nanobubbles from being followed for longer periods.

To evaluate the stability over a longer period of time, a freshly prepared sample of ec-H₂O sub 450 nm was measured at an interval of 1 day using the NanoSight. The syringe was filled with the sample immediately after production and remained undisturbed and sealed for 5 days. Each day, more of the sample was measured. Daily measurements confirmed that the size did not change significantly. Although the mean nanobubble size increased from 96 nm on the first day to 120 nm on the third day, the concentration decreased significantly over this time, as shown in Figure 3b and Table S1. This indicates that the size and concentration of nanobubbles are maintained for approximately 24 h after production.

Note that the ec-H₂O Nanoclean cell has platinum-coated electrodes, which degrade over time. Therefore, it is possible that platinum particles are being liberated as nanoparticles and that these nanoparticles may influence our data and/or the cleaning processes. A calculation was performed to determine if the concentration of nanoparticles observed could be due to platinum particles based on the known rate at which the electrodes degrade. This calculation revealed that if the nanoparticles produced were solely platinum then all of the platinum in the ec-H₂O Nanoclean cell module would be exhausted after less than 30 L had been passed through the unit. This is inconsistent with the lifetime of the unit, which shows that many thousands of liters are produced before the platinum is substantially removed. Furthermore, our experiments conducted over a period of more than a year (and after hundreds of liters have been produced) show a consistent production of nanoparticles in both size and concentration. Additionally, it is observed that in a given sample the concentration of nanoparticles observed decreases steadily over a period of days but the size remains rather consistent. This is not consistent with the presence of platinum particles,

which we would expect to be either stable or grow substantially in size before settling in solution. We can therefore discount the possibility that the electrolysis process is producing a stream of platinum nanoparticles that are interfering in our experiments.

At normal pH values, it has long been known that bubbles have a negative surface charge. This is attributed to the preferential adsorption of hydroxide ions.^{32,33} The zeta potential of ec-H₂O sub 450 nm nanobubbles as a function of pH is shown in Figure 4. The isoelectric point (iep) is

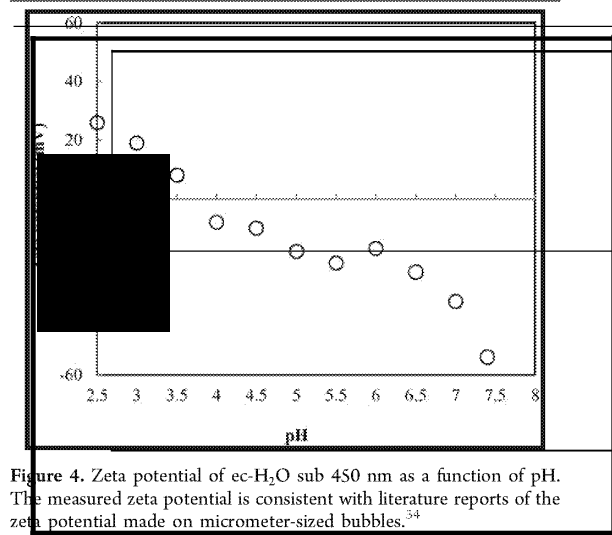


Figure 4. Zeta potential of ec-H₂O sub 450 nm as a function of pH. The measured zeta potential is consistent with literature reports of the zeta potential made on micrometer-sized bubbles.³⁴

observed at a pH of ~3.7. This is consistent with the measurement of the zeta potential on larger bubbles, as is the magnitude of the zeta potential in a solution of this concentration of NaCl.³⁴

Cleaning of Contaminated Surfaces with Nanobubbles. The first mode of cleaning that we investigated was whether ec-H₂O could remove BSA and lysozyme from a hydrophilic surface. The proteins (BSA and lysozyme) were used as model contaminants because they had been the subject of earlier cleaning experiments using surface nanobubbles.^{15,17}

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DOI: 10.1021/acs.langmuir.6b01004
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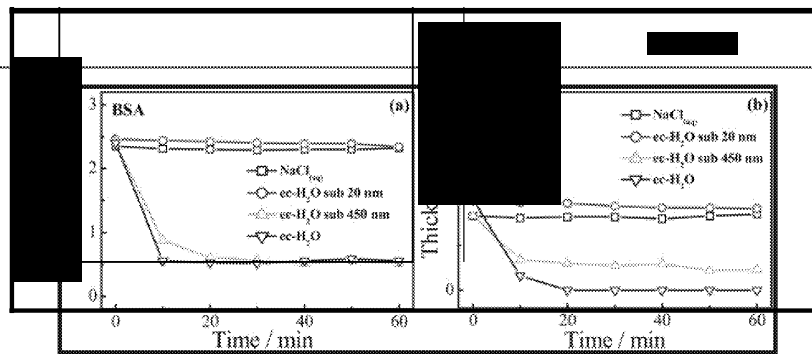


Figure 5. (a) Exposure of BSA previously adsorbed on a hydrophilic silicon wafer to both nanobubble and control solutions. (b) Exposure of lysozyme previously adsorbed on a hydrophilic silicon wafer to both nanobubble and control solutions.

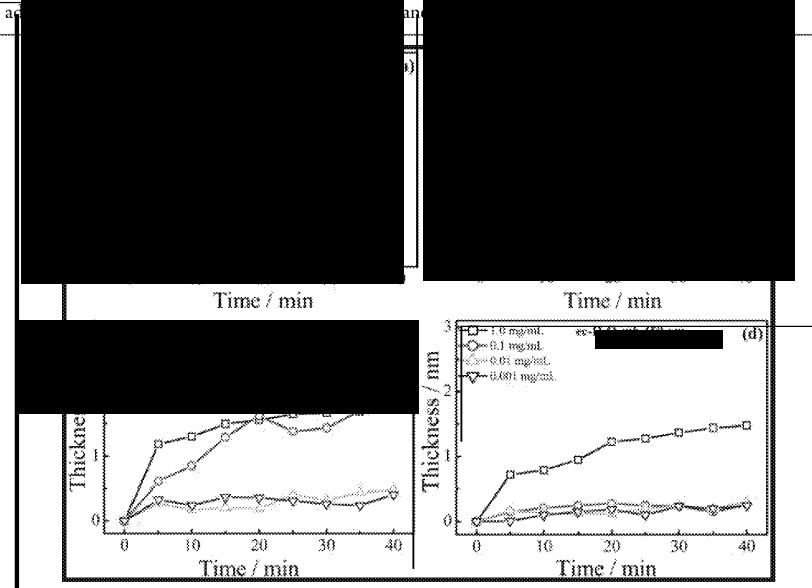


Figure 6. Adsorption inhibition study performed using ellipsometry to measure the thickness of adsorbed BSA to a hydrophilic silicon wafer as a function of time. Here, the thickness of adsorbed protein was measured in air. (a) BSA adsorption from NaCl solution (2.1 mM). (b) BSA adsorption from ec-H₂O solution. (c) BSA adsorption from ec-H₂O sub 20 nm solution. (d) BSA adsorption from ec-H₂O sub 450 nm solution.

BSA at a concentration of 1.0 mg/mL dissolved in NaCl solution (2.1 mM) was allowed to adsorb to a clean hydrophilic silicon wafer for 60 min with a wet thickness of ~ 2.4 nm in aqueous solution. Then the surface was treated with different types of solutions, and the thickness of the adsorbed BSA layer was measured as a function of time of treatment by ellipsometry in situ in NaCl solution (2.1 mM). To avoid the interference of nanobubbles in the ellipsometry measurements for ec-H₂O, ec-H₂O sub 450 nm, and ec-H₂O sub 20 nm solutions, the relevant solutions were replaced by NaCl solution every 10 min to measure the protein layer thickness. It was found that ec-H₂O and ec-H₂O sub 450 nm cleaned the surface with similar effectiveness, whereas the control NaCl solution and ec-H₂O sub 20 nm solution had no effect. (Figure 5). This fact suggests that the nanobubbles in the size range between 20 and 450 nm contribute to the cleaning effect. This is consistent with the results observed in Figures 2 and 3, where the size of the nanobubbles is distributed around 90 nm. Likewise, lysozyme molecules were adsorbed onto the hydrophilic silicon wafer surface from a solution of 1.0 mg/mL lysozyme for 60 min, producing a wet thickness of ~ 1 nm. Lysozyme can also be removed by ec-H₂O and ec-H₂O sub 450 nm solutions. In contrast, the NaCl solution and ec-H₂O sub 20 nm solution

had no cleaning effect on the lysozyme-coated silicon wafer surface.

Preventing Surface Contamination. It is known that effective cleaning often requires mechanical agitation. This agitation will release contaminants from the surface; however, for the cleaning to be effective, it is important that the material is not redeposited. The suspension of soil in the cleaning fluid and the removal of the fluid are important in this respect. In situations where the cleaning process involves short periods of exposure to cleaning solutions of a few seconds, we considered the possibility that the cleaning effect of both detergents and nanobubbles in commercial applications may be achieved by stabilizing contaminants in solution that have been detached by mechanical scrubbing rather than removing contaminants from the surface. Therefore, we decided to evaluate whether ec-H₂O can prevent the deposition of material to a surface. To do so, we dissolved different concentrations of BSA in solutions and observed the adsorption of BSA to a surface over time. If the amount of material adsorbing to the surface is reduced, then this would indicate the effective prevention of deposition of the contaminant from solution. Figure 6 shows the adsorption of BSA over a 40 min period from different types of solutions. It is clear that the adsorption from ec-H₂O and NaCl is the same at

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DOI: 10.1021/acs.langmuir.6b01004
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