This work presents in situ imaging of synthesized boehmite (γ-AlOOH) particles ranging from 20 to 100 nm, suspended in liquid, in a vacuum compatible microfluidic sample holder using a scanning electron microscopy (SEM) under the high vacuum mode and highlights the advantage of in situ liquid imaging of colloids. Nanometer-sized boehmite particles in high-level radioactive wastes at the Hanford site are known to be difficult to dissolve and cause rheological problems for processing in the nuclear waste treatment plant. Therefore, it is important to characterize boehmite particles and understand how they form aggregates in the liquid state. Several technical advancements are made to optimize in situ liquid SEM chemical imaging resulting in the improved ability to obtain secondary electron (SE), backscattered electron (BSE) images, and energy dispersive X-ray spectroscopy (EDX) spectra. Moreover, our results show mixed particles could be studied and identified based on the particle shape and elemental composition using in situ SEM imaging and EDX. Thus, we provide a new and improved approach to observe the evolution of particle dispersion and stability in liquid under conditions similar to those in the waste tank.

**KEYWORDS**
boehmite, BSE imaging, EDX, in situ liquid SEM, SALVI, SE imaging

**1 | INTRODUCTION**

The interactions between colloidal particles in liquid suspensions play an important role in the property and behavior of many materials with diverse applications fundamentally and at the industrial scale. Although the Deryaguin-Landau-Verwey-Overbeek theory gives good prediction of particle interactions of single component monodisperse particles, complex colloidal systems that are unstable are less understood. One example of such colloidal systems is the Hanford tank waste slurry consisting of radiological mixtures of soluble and insoluble solids with high salt concentrations. Boehmite (γ-AlOOH) has been identified as a key component in the waste treatment process and used as a surrogate model system in understanding real waste rheology and its implications in waste processes. Atomic force microscopy (AFM) has been widely used to directly measure the forces between particles and surfaces; however, it is difficult when it comes to nanometer-sized particles. In Hanford nuclear wastes, the most representative size of boehmite particles is 100 nm or less. Thus, in situ imaging with field emission scanning electron microscope (FESEM) is used to address this challenge.

In situ electron microscopy is attractive in studying biological samples, ionic liquids, or spent nuclear materials. Several approaches are used to study liquid in SEM. Atmospheric inverted SEM was developed to study biological samples. Another major instrument development is the environmental SEM (ESEM). The increasingly more attractive way is using vacuum compatible liquid cells. Analysis of liquid samples using either SEM or transmission electron microscopy (TEM) demands cell compatibility to high vacuum on the order of $10^{-6}$ Torr. Wet cell SEM was commercially available with products from SPI and QuantomiX. Only backscattered electron (BSE) imaging was possible in these commercial cells. Silicon nitride (SiN) or silicon dioxide (SiO$_2$)
is a popular choice for window materials due to the high electron transparency in wet cells developed for SEM.7

There has been a surging interest in liquid cells in electron microscopy in recent years. Many more works have been reported in TEM than SEM. Several recent reviews cover this subject.7–9 Besides SiN membranes,10–13 graphene has emerged as an optimal window material to overcome issues using SiN recently.9,14–17 Generally, fully enclosed liquid cells are used in SEM and TEM. Besides the windowless design presented in this work, a recent effort showed a polymer generated dome nanoreactor for TEM study of nanoparticles.18

We invented a vacuum-compatible microfluidic interface, namely a system for analysis at the liquid vacuum interface (SALVI), and enabled analysis in situ liquid SEM.12,13 Compared with the existing wet cell SEM approaches,19–21 the liquid surface is probed directly by the primary electron beam, because the microfluidic cell is partially open to vacuum with micrometer-sized apertures. In addition, the beam effect and memory effect can be minimized by flowing the liquid.12,17 We demonstrate optimization of in situ liquid SEM for suspended boehmite particle imaging and elemental analysis in this work. Reproducible secondary electron (SE) imaging, BSE imaging, and energy dispersive X-ray spectroscopy (EDX) analysis were achieved after improving the overall workflow and optimizing the accelerating voltage. A simulated particle mixture in liquid was attested. Our results show particle identification in particle morphology and elemental composition. This work presents a valid tool for investigating complex particles in the liquid environment.

2 | EXPERIMENTAL

Rhombic plates of boehmite were prepared in the range of tens of nm to 100 nm according to modified literature procedures.22,23 The crystal structure was confirmed using XRD (Figure S1), IR (Figure S2), and SEM (Figure S3).

Two FEI Quanta 3DFIB-SEM instruments were used for dry and liquid analysis in the high vacuum mode. Figure S3 depicts the SEM characterization of the dry particles studied in this work, including Ag NPs, ZnO NPs, and γ-Al2O3. Detailed SEM procedure and parameters used in acquiring SE/BSE images and EDX spectra were detailed elsewhere.24 The main chamber vacuum was maintained generally at 1 to 8 × 10−6 Torr during experiments.

The fabrication details of the vacuum compatible microfluidic reactor or SALVI were described previously.12,25,26 Soft lithography was used to make a 200 to 500-μm-wide by 300-μm-deep channel on a silicon wafer as the microfluidic mold. The channel width did not have an effect on the analysis. However, wider channel was convenient for making more spaced apertures during the experiment. More details are provided in SI.

3 | RESULTS AND DISCUSSION

3.1 | Improvement in workflow

Several technical advancements have been made to optimize in situ liquid SEM imaging of particles in this effort. First, Figure S4 depicts the SALVI setup picture inside the SEM main chamber in two different instruments. A gold (Au)-coated device was used in the radiological SEM-FIB instrument (Figure S4a) on campus. Two devices were loaded for analysis at the same time. Copper tape was used to form the contact between the device and the stage to reduce charging during analysis.12,13 Since Au may be a component of a sample and interfere elemental analysis, carbon coating was chosen and deemed advantageous for broad applications of liquid samples.24 Figure S4b shows a carbon-coated device secured with carbon tape on the stage in another SEM-FIB instrument. In situ liquid SEM imaging and EDX analysis were conducted in three SEM-FIB instruments and showed good performance as discussed herein.

Figure 1A depicts 12 fibbed holes on the SiN membrane prior to in situ liquid SEM imaging. Figure 1B gives a closer look of an aperture with 840.7-nm diameter. Using the gallium FIB, a series of 1-μm-diameter holes were milled with 100-μm distance apart prior to the in situ liquid SEM analysis. In this example, 12 holes were prepared in this particular experiment.

Using the gallium focused ion beam (FIB), a series of 2-μm-diameter holes were milled with 100-μm distance apart prior to the in situ liquid SEM analysis. In this example, 12 holes were prepared in this particular experiment.

Second, the operation steps are improved. In our first illustration of in situ liquid SEM using SALVI, the 2-μm-diameter hole was fabricated using SEM-FIB on the SiN membrane prior to device assembly.12,26 Although this is doable, it requires some skills under the optical microscope to align the SiN and the microchannel. Another shortcoming is the limitation of the number of observation windows or apertures in an experiment. When an SEM is equipped with FIB, it is more convenient and efficient to mill the apertures after a device is made. Figure S4 shows a row of holes on the SiN membrane milled by the Ga gun along the channel right before an analysis. Based on our experience, it is safe to make several to a dozen or more holes in an experiment. The distance between holes is set at 100 μm apart to ensure that the SiN is not weakened too much.

The third change is to reduce the observation window from 2 μm to approximately 1 μm because of the fine focus and magnification power of SEM. An additional benefit of using a smaller aperture is to decrease loss of water vapor under high vacuum.12,26 Much of the data presented here were obtained using the aperture smaller than 1 μm. A detailed step-by-step procedure of in situ liquid SEM was recently published elsewhere24 to provide technical guidance. We focus on analytical capability development in this paper and show results in optimized SE imaging, BSE imaging, and sensitive EDX analysis of particles dispersed in liquid.

3.2 | Optimizing the accelerating voltage

An important factor in obtaining high quality SEM images is the accelerating voltage. The SEM resolution is affected by many factors, such as the size of the area being probed, the size and energy of the primary beam, specimen composition, or the type of signals being
FIGURE 1  A. A series of SEM-FIB milled holes with a diameter of approximately 1 μm imaged prior to the liquid SEM analysis and B, a close look of an aperture with 840.7-nm diameter. [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 2  (Color online) A comparison of SE and BSE images of AlOHH under different HV: 5 kV (A and B); 8 kV (C and D); and 10 kV (E and F). These images were acquired using 100 000 magnification. The working distance was 8.1 mm in A and B, 9.9 mm in C and D, and 5 mm in E and F.
imaged. Although SEM cannot match TEM in terms of resolution, SEM excels in the depth of field. This is generally handled by adjusting the working distance and accelerating voltage. Emission signals of SEs with energy less than 50 eV are more widely used in SEM. The penetration and interaction volume were long discussed in solid targets.27 The penetration depth and interaction volume is a direct function of energy, the higher the energy the deeper the depth. However, it is not necessarily true that the higher the energy the better the images. Some examples were given in soft materials like mammalian cells or tissues using SEM.28,29 As AlOOH particles in liquid belong to soft materials, the accelerating high voltage (HV) is optimized to achieve satisfactory results.

Al is a light element with an excitation energy of 163 to 169 eV.30 Figure 2 depicts SE and BSE image comparison under three different conditions. In the SE image, brighter regions correspond to hills and dark regions valleys. In the BSE image, bright regions correspond to the presence of heavier elements. The particle images are unclear in either SE or BSE when applying 5 kV. This condition is good for soft materials like cells or polymers in surface imaging,28,29 but not suitable for Al. The SE and BSE images are much improved at 8 kV. Figure 2C shows the location of boehmite particles in bright contrast in the aperture. In this case, Al is imaged at the same location in Figure 2E as in Figure 2C. Given there is only one atomic number difference between Al in boehmite and Si in the SiN membrane, the contrast in the BSE image (Figure 2C) is reasonable. When the voltage was increased to 10 kV, the imaging is not as good as the 8-kV results. It is worth noting that our approach can provide both SE and BSE imaging compared with the existing wet cell SEM.31 The latter only offers BSE images.

Higher acceleration voltage leads to more primary electrons penetrating deeper into the material and reduces SE yield and surface details. Although BSEs come from below the surface, 8 kV seems to provide the best images among the relatively low and high accelerating voltage conditions. The topic of the information depth of SE and BSE in a specimen has many considerations. Typically, SE reflects...
surface-bound properties of the top few nanometers of the material, and it is independent of the landing energy of excited electrons. BSE can provide information at certain subsurface depth. The contrast in the image reflects contributions from the material composition, crystalline structure, and even electronic structure. A recent systematic BSE study of steel surface offers recommendations for routine estimations of BSE information depth on solid samples in the energy range of keV. More detailed analysis and comparison among analytical equations for information depth and the depth distribution function approach (e.g., Casino Software) may be needed for future endeavor to fully understand the electron beam effect on image contrast in soft liquid materials.

We also compared the SE images of a 100 \( \mu \)g/mL boehmite in water sample using 1-\( \mu \)m-sized windows under 5, 8, and 10 kV with two different magnifications, 80 000 and 100 000 (Figure S6). The SE image acquired at 8 kV offers best imaging conditions for boehmite as discussed earlier in Figure 2. Due to the outstanding finely focused electron beam in SEM, 2 \( \mu \)m is not necessary in the liquid analysis as what we perceived originally for liquid SEM and time-of-flight secondary ion mass spectrometry using SALVI. Using a smaller aperture does not sacrifice the SE and BSE image quality, yet further reduces loss of water. However, it is the operator’s choice to use 1 or 2-\( \mu \)m diameter apertures in an experiment.

In a recent liquid SEM experiment using sealed cells, a Photochips Poseidon 200 fluidic cell holder with SiN windows that were 40 nm thick was illustrated in a Hitachi FE-FIB-SEM and Hitachi FE-SEM. Comparison of the images of gold nanoparticles acquired at 4 and 25 kV showed that higher voltage would give brighter SE contrast. Interpretation of liquid cell SEM experiments need to consider contributions from the dielectric membranes, fluid used, type of samples, and the potential beam interactions.

### 3.3 | SE and BSE imaging and EDX analysis

As a reliable characterization tool, image and EDX analysis reproducibility is highly imperative. Figure 3 shows SE imaging of AlOOH particles in water taken consecutively in six apertures. The particle shape and size are consistent among these observation windows. In each hole, multiple locations were randomly selected to snip EDX spectra for local elemental composition. Figure 3G-I gives an example of three EDX spectra collected in three corresponding locations.

**FIGURE 4** (Color online) A, SE and B, BSE images of mixture consisting of 100 \( \mu \)g/mL AlOOH with trace ZnO and Ag NP in water mixture taken under 8-kV HV using 200 000 magnification with a 10.3-mm working distance; C, a EDX spectrum taken inside the aperture illustrated in the green box in A showing elemental identification of Al, O, and Zn; and D, a EDX spectrum taken inside the red box showing elemental identification of Al, O, Zn, and Ag. The vacuum was maintained at 4.4 \( \times \) 10\(^{-6}\) Torr. [Colour figure can be viewed at wileyonlinelibrary.com]
highlighted in Hole 5. Strong O and Al signals validate the observation of Al and O from AlOOH and water. The carbon peak comes from the graphite coating. The Si and N signals are mostly from the analysis volume of SEM.\textsuperscript{12,26} Reproducible images of boehmite over an hour of measurement also suggests that the measurement time scale is relatively short compared with the aggregation time that is known to occur in boehmite particles. Therefore, in situ liquid SEM is a viable approach to study relatively unstable particles like boehmite.

Figure S7 provides additional EDX spectra from Hole 3 in Figure 3C. Thus, reproducible in situ SEM imaging and EDX analysis were achieved using the optimized HV conditions. Similar experiments were repeated in multiple months with different devices and samples containing boehmite. The optimized conditions produced consistent results, showing the robustness of the approach.

3.4 | Identification of mixed particles

Most particles exist in chemically complex mixtures not as a single component. A simulated mixture consisting of primarily AlOOH particles and trace Ag NPs and ZnO NPs were examined using in situ liquid SEM. The channel was flushed with 100 $\mu$g/mL Ag NPs in DI water followed with DI water rinse and repeated with filling up with 100 $\mu$g/mL ZnO NPs in water and rinsing before filling up again with AlOOH particles in water. Figure 4 shows the SE and BSE images of three types of particles. The morphology and shape of Ag, ZnO, and AlOOH are different in the dry form (Figure S3). The images of these particles in water are not as sharp as those deposited on the Si wafer. However, the contrast and shape difference are visible. The seemingly less desirable images do not necessarily suggest a “bad” result; this may be a combined effect of local drying\textsuperscript{13} and the interactions of water surrounding the particle. Beam damage is less significant at the surface due to liquid diffusion.\textsuperscript{26,36} For instance, our preliminary analysis of boehmite particles using ToF-SIMS suggests water cluster and cluster ion formation when boehmite particles are suspended in water, resulting in chemical composition changes, which are likely directly reflected in the shape and morphological variations.

To validate the particle type determination based on shape and size, EDX spectra were taken at different locations. Figure 4C is the EDX spectrum acquired at the top left corner of the aperture. ZnO NP and AlOOH particles were more abundant at this location. In the lower corner, Ag NPs were more visible as confirmed in the EDX spectrum in Figure 4D. Some Ga signals were observed, likely due to the residual from the FIB process. The source of Cd was probably from the Ag NP and ZnO NP synthesis. Image analysis of boehmite inter-particle distance is presented in a companion paper.

4 | CONCLUSIONS

In conclusion, in situ liquid SEM imaging and analysis of particles in liquid in the high vacuum mode is improved in the following aspects: (a) the usage of carbon-coated and Au-coated devices to reduce charging; (b) dynamic SEM/FIB milling to provide more observation windows in a given experiment; (c) smaller observation apertures because of the fine focus of SEM; (d) optimized SE and BSE imaging for Al and other light elements; (e) validated reproducibility in imaging and EDX analysis; and (f) interrogation of mixed particles. The reported optimization and enhancement enable direct observation of particle evolution in morphology, aggregation, and composition in liquid, thus providing a reliable way to study particles and particle systems in diverse applications in the future.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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