

# High-Precision Acoustic Transfer of Buffered Solutions Containing Surfactant and Protein for Miniaturized Biological Assays

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## 1. ABSTRACT

Surfactants and proteins are key components present in aqueous solutions used in biological assays and have historically been problematic to dispense with high-speed tip- or nozzle-based liquid handling instrumentation. In the case of buffered solutions containing surfactants, due to their low surface tension these fluids have a high tendency to leak out of tips or nozzles. Furthermore, buffered solutions containing surfactants often contain bubbles that can become entrained or trapped in the tips or nozzles of standard liquid handling instrumentation. Dissolved protein can also serve to introduce bubbles into traditional aqueous dispensing. Because of these difficulties, there is clearly a need to extend the nozzle-less fluid transfer technology of acoustic droplet ejection (ADE) to robustly handle fluids containing surfactants and proteins.

Acoustic droplet ejection (ADE) is a unique liquid transfer technology that eliminates the use of tips or nozzles and has been proven to dispense more accurately and precisely in the low nanoliter regime with DMSO as compared to tip-based liquid handling. When transferring solutions containing protein using ADE, the interaction of the protein at the fluid-well interface can cause a highly tilted or an irregular meniscus (often compounded by the centrifugation of well plates) which can lead to inaccurate and imprecise dispensing in an acoustic-based liquid handling instrument that was not modified to deal with source well meniscus variation. The work described here extends the capability of the Echo® 555 liquid handler (Labcyte Inc.) which utilizes ADE technology to accurately and precisely dispense buffered solutions containing surfactant or protein or both surfactant and protein. Performance with these difficult solutions is now comparable to that of DMSO and of aqueous buffer solutions containing moderate salt concentrations.

### Acoustic Droplet Ejection: Move Liquids with Sound™

Acoustic Droplet Ejection (ADE) uses focused ultrasonic energy to eject small droplets from the surface of a liquid (Fig. 1). The technology can be used to eject droplets smaller than one microliter and as large as ten microliters. Larger volumes can be transferred as multiple drops. There is no contact between the ejection mechanism and the ejected sample. This technology is especially well suited to biological applications in which volume and/or positional precision are critical, and in which cross-contamination of samples can interfere with interpretation of the results. The acoustic mechanism eliminates the need for pipette tips, pin tools or nozzles.

Figure 1. Stroboscopic image of ADE and an Echo 500 series liquid handler that uses ADE.



## 2. METHODS & MATERIALS

### REAL-TIME ACOUSTIC MONITORING

The piezoelectric acoustic transducer used in the Echo 500 series liquid handlers both transmits and receives acoustic signals. We have developed both new methods and improved software algorithms to utilize real-time acoustic measurements and responsive acoustic pulse generation to overcome the challenges of transferring solutions that have low surface tension (e.g. surfactants) or unstable menisci (e.g. protein solutions). Sound waves are emitted into the sample at lower energy than is required to eject a droplet, thereby perturbing the fluid meniscus. A second acoustic pulse is then emitted to reflect off of the perturbed fluid surface, and the pulse signal is digitally captured.

Real-time analysis of this reflected signal allows the dynamic calculation of the optimum acoustic energy required to eject a droplet on a well-to-well level. The system then automatically adjusts the acoustic energy to this optimum level before a transfer event. The process is performed in real time immediately prior to the transfer from each well in order to correct for actual fluid and meniscus conditions.

One of the main advantages of real time dynamic measurement of fluid properties is the capability to reproducibly transfer fluids of varying surface tension allowing the user to transfer different concentrations of buffered solutions containing surfactants and proteins on a well-to-well basis.

## EXPERIMENTAL PROTOCOL

To test transfer performance of buffered solutions containing surfactant, Triton X-100 and sodium dodecyl sulfate (SDS) were diluted into 1x phosphate-buffered saline (PBS) with 0.15 mM sodium fluorescein to a wide range of final concentrations spanning from 0.1% to 200% of the critical micelle concentration (CMC) of the surfactant. ADE was then used to dispense 50 nL of these solutions from a 384-well polypropylene source plate, using a range of source well fill volumes, into 384-well destination plates (Greiner Bio-one). The destination plates were then filled with 50 µL of 10 mM NaOH, centrifuged, incubated for 30 minutes, and the fluorescent signal was measured from each well on a 2100 EnVision fluorescence reader (PerkinElmer). Relative fluorescence units (RFUs) were converted to volumes using a standard curve.

To test transfer performance of buffered solutions containing protein, the above experimental protocol was followed using either 10% fetal calf serum or a range of protein concentrations from 0.1 – 10 mg/mL of bovine serum albumin (BSA). Transfer of buffered solutions containing both surfactant and protein was also tested with the above protocol. Finally, the concentration of surfactant (SDS and Triton X-100) present in the buffered solution was varied from 25% to 200% of the critical micelle concentration (CMC) to create a "torture" plate which was also tested using the above protocol.

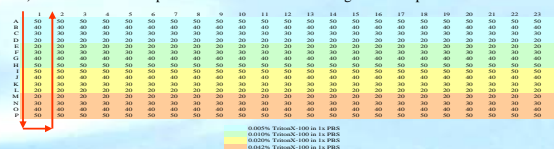


Table 1. Plate map for varied surfactant concentration and source well fill volume on the same plate to create a "torture" plate. The red arrows indicate the transfer path of the transducer for the ADE transfer process.

## 3. RESULTS

### TRANSFERS WITH BUFFERED SOLUTIONS CONTAINING SURFACTANT

Results yielded average transfer precision of 2.1% CV for both Triton X-100 and SDS across a wide range of concentrations and source well volumes (see Fig. 2. below).

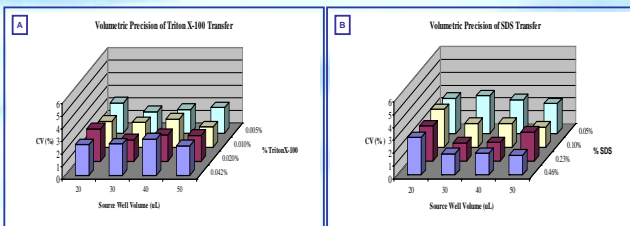


Figure 2. Volumetric precision values for (A) Triton X-100 and (B) SDS in the bar charts above. For each source well fill volume and concentration, 50 nL of buffered surfactant solution was transferred from a 384-well plate. Each CV was calculated from 384 replicates.

### TRANSFERS WITH BUFFERED SOLUTIONS CONTAINING PROTEIN

Results yielded average transfer precision of 2.3% for buffer containing 10% FCS in 1x PBS across the whole range of fill volumes from 20, 30, 40 and 50 µL. In this case the CV value was determined from transfers of 5,760 replicates. Furthermore, for these experiments, the utilization of the improved algorithms enabled automatic flagging of non-transferred wells and a transfer efficiency of 99.975%.

### TRANSFERS WITH BUFFERED SOLUTIONS CONTAINING SURFACTANT & PROTEIN

Typically, biological assays are comprised of buffered solutions containing both surfactant and protein. By using these new methods with the improved algorithms for signal processing, signal recognition and real time measurement, excellent precision (average = 3.0%) and accuracy (average = 4.8%) were obtained (see Fig. 3. below).

### TRANSFERS OF BUFFERED SOLUTIONS WITH VARIED SURFACTANT CONCENTRATION

Finally, a "torture" plate of different wells filled with different concentrations of surfactant (ranging from 25% to 200% of the CMC) in buffered solution was tested and produced both excellent precision (average = 3.8%) and accuracy (average = 2.1%) for 50 nL transfers (see Fig. 4. below).

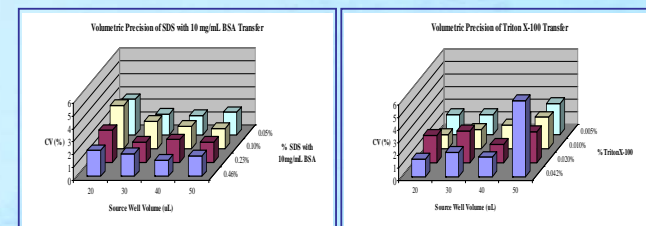


Figure 3. Volumetric precision values for varying SDS concentration and 10 mg/mL BSA in 1x PBS above. For each source well fill volume and concentration, 50 nL of buffered surfactant solution was transferred from a 384-well plate. Each CV was calculated from 384 replicates.

Figure 4. Volumetric precision values for varying Triton X-100 concentration in 1x PBS and source well fill volume above. For each source well fill volume and concentration, 50 nL of buffered surfactant solution was transferred from a 384-well plate. Each CV was calculated from 24 replicates.

## 4. CONCLUSIONS

- New enhancements to the Echo software have enabled excellent volumetric precision and accuracy for the transfer of surfactants, serum, proteins and other fluids that are difficult to transfer by most liquid handling technologies.
- New methods for acoustic droplet ejection along with improvements to signal recognition algorithms greatly improve transfer performance from unstable menisci in protein solutions.
- Dynamic monitoring of acoustic properties of fluids on a well-to-well basis allows for accurate dispensing of plates filled with a variety of fluids with varied surface tension properties.
- ADE offers a touch-less transfer process that enables miniaturization of biological assay set up, reduced costs (no pipette tips) and minimized reagent waste. This work has been also been extended to qPCR.