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ACOUSTIC LIQUID HANDLING MASS SPECTROMETRY: A NEW MILESTONE IN LABORATORIES

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REVIEW ARTICLE

ABSTRACT

By ruling out other sample injection systems, new acoustic liquid sample introduction technique is developed. The acoustic droplet ejection occurs by focusing sound wave to propel droplets of liquids upward from a source location to a destination which further get ionized and forwarded to mass spectrometer. Smaller amount of sample volume like 2.5 nano litre droplet splits into hundreds of even smaller droplets like femtolitres, which is used to generate the data. Acoustic Droplet Ejection (ADE) and its wide application in mass spectrometry generated full library screen in excess of 2 million compounds in less than six weeks with good precision and accuracy. So far the most important, achieving high scientific impact, ADE is a completely touchless transfer method that shall provide a new milestone in analytical world.

Key words: Acoustic Droplet Ejection, Mass Spectrometry, Echo 650 LIQUID HANDLER

INTRODUCTION

Several laboratory techniques are available to handle liquid samples but dispensing liquids with high precision and accuracy is troublesome. Like in biological assays shrinking to micro litre volumes, it's dispensing and handling was much difficult based on air displacement and was also less accurate as well as unpredictable.

The advancement of research in life sciences through better revealing systems necessitate essential changes in handling liquids and preparing samples. The traditional tip-based concept was able to achieve various throughput but they also create obstacles in achieving results at reasonable costs. It may also result into contamination and variable allot volumes, prolonged cleanup, calibration and setup processes involving large volumes of costly reagents. Acoustic droplet ejection (ADE) handles these issues by moving liquid with acoustic energy. Acoustic droplet ejection (ADE) is a new technique widely accepted as "state-of-the-art for handling liquid solutions as high-throughput screening for drug discovery as well as for other applications". This new emerging technology utilizes the sound energy to transfer liquids with touchless features having no pipette tips or pin tools. The purpose of using sound waves is to maintain the ejection of similar droplets of liquid under study and transfer to a microplate or any other surface, i.e., from source location to a destination, for its various uses (Stearns et al, 2018). The basic property relies on the fact that the sound waves are easier to be insured through transducers or radiofrequency generators which helps in providing high precision as well as accuracy to the acoustic signals. Also, the coefficient of variability of liquid sample transfers using acoustic droplet ejection has been reported to be much lower compared to other techniques (Comley, 2004). An attractive feature of ADE is that it is not restricted to limited range of liquid samples and also no calibration is required while dealing with different liquids.

The journey of this technique begins, in 1920s by scientists Robert Wood and Alfred Loomis (at laboratory in Tuxedo Park, New York), with the discovery that an acoustic energy when projected through a liquid from low surface caused a vent like structure at the surface as a result of which emerged droplet was shot upward. The acoustic energy uses high-intensity acoustic beams which results "erupting liquid droplets like a miniature volcano." Further in the 1950s and early 1960s acoustic signals applications to form minute droplets in liquid was studied and used in commercial nebulizers to create mists of medications for ingestion. The actual work starts with the prologue when a low-intensity process that centered and throbbed acoustic energy to produce a single "drop-on-demand" was formulated in 1970s (Tulaikova, et al, 2015).

The present study deals with the application of acoustic signals to create high throughput for various liquid handling-based applications.

Literature review

The substantial research in the field of acoustic droplet ejection (ADE) has been practiced since a decade but the involvement of ADE in various applications of life science processes has made a complement in recent years (Hadimioglu et al, 2015). Ejection of fluids acoustically has been applied for various applications like loading of sample into mass spectrometer for precise and accurate output (Ellson et al, 2003), protein crystallography (Soares et al, 2011), integration multiple libraries in DMSO (Grant et al, 2009; Griffith et al, 2012; Zaragoza-Sundqvist et al, 2009), cell patterning (Demirci, 2007; Fang et al, 2012). The coupling of Echo 525 liquid handler with TXTL has made a revolutionary remark in the study of protein synthesis (Balley et al, 2018). The use of sound energy transfers contamination-free reagents without compromising their quality and concentrations to nozzles or tips. The flow rate is maintained up to 5000 nL/s, which reduces reagent cost with high precision and accuracy that utilizes the volume for processes involved in transcription and translation study. "Metagenomic next-generation sequencing (mNGS)" is a functional device involving acoustic Echo 525 to dispense volume with high precision and accuracy providing several unique features like miniaturization, automation, cheap and rapid (Madeline et al, 2018). ADE, a recent touch-less technology, drive droplets from diverse solutions with high precision (Ellson et al. 2003; Shieh et al. 2006; Harris et al. 2008; Rudnicki et al. 2009) and has applications in various fields including drug delivery. Achim in 2006 discovers an approach for flexible chip-based microfluidic system with exclusive properties adopted by reviewing practicality contriving the system.

In the scientific field, the conclusion drawn on the basis of Mass spectrometric data has made an advanced learning arena where access of analyzed sample in the form of ions can also be carried out using sound waves. Mass spectrometry (MS) utilizes the concept of ionization of molecules, their electro-magnetization ensembling to provide mass-to-charge ratios to recognize target species based on molecular mass. The use of ultrasounds in generating mass spectra (MS) is a new technique that emerges with various characteristics features. Labcyte Echo® acoustic liquid handler is utilized to eject 2.5 nL droplets directly from 384-well test plate into an “Open Port Probe” (OPP) sampling edge. Captured droplets are diluted into a continuous flow of solvent and the OPP liquid flow is drew out to electrode where ionization of sample is carried out using standard electrospray before entering a “Sciex 5500 QTrap mass spectrometer”. This new ADE-OPP platform leverages the high speed and excellent accuracy of ADE liquid handling technology into sample delivery for mass spectrometry. The samples further can be analyzed for different applications like notifying progress of a reaction, degradation of a compounds, detection of impurity to heavy matrix systems, etc. Huang et al, 2012 explored the technique where liquid samples are nebulized after lodging on a surface of piezoelectric as a lean haze with the generation of acoustic signals across that surface. It produces droplets having less kinetic energy that results into easy and efficient uptake to the capillary of the inlet. A different study reported that the use of acoustic signals is very reformative in ESI that can analyze both ions and neutral species having high ionization efficiency than SAWN (Snijder et al, 2013). The evolution of “dynamic fluid analysis”, as outlined by Sackmann et al., 2015 produces auto monitor and calibrate the system. Recently, the team of Scientist Jonathan M Wingfield at AstraZeneca, Stockport, United Kingdom is actively involved in exploring various other applications of Acoustic Droplet Ejection Technique.

Working of Acoustic technology

ADE utilizes sound energy to raise minute droplets of fluids from a source plate to a target site. The source plate is kept fixed whereas the target plate is dynamic to permit the transfer of solutions from wells of the source plate to the wells of the target plate lying in an inverted position (Olechno et al, 2016). The method doesn’t involve any auxiliary consumable except micro plates (Olechno et al, 2016), however, it accelerates the process by annulling cleanup steps having the potential to make assay-ready plates (Turmel et al, 2010). The modern ADE technique is featured with the fact that it utilizes low intensity sound signals that are focused at the meniscus, resulted into the formation of a mound at the surface of liquid. Further, supply of even smaller amount of energy creates the droplet which is propelled to move upward on to a target having inverted position with unique accuracy as well as precision.

The technique employed in ADE belongs to entirely touchless transport methods. Conventionally, the term “noncontact transfer” was applied to the apparatus which channelize the liquid that doesn’t come in contact with the solid or liquid surfaces of the target plate but was injected straight into the well. Since, the conventional method was based on the transfer of liquid from source plate to destination plate having contact between the tips or nozzle and the sample being transferred which affect the sample via contamination and thus was ruled out in modern ADE technique. This eradicates the possibility of solute being absorbed by the transference device while at the same time forbidding leachates, that could affect the analysis through contamination of the transferred liquid (McDonald et al, 2008; Belaiche et al, 2009). The ATS Acoustic Liquid Dispenser of EDC tuned up the sound signals to generate droplets having volume in the range of 1nl–20nl. However, with fine tuning, it is probable to distribute 25–50 nl reproducibly.

An Echo® acoustic liquid handler is customized to make a convention phase which displaces a 384-well microplate to locate on the piezoelectric transducer for sound signals (Figure 1). An OPP sampling port is positioned above the plate so that minute droplets coming out on the basis of ADE can be quantitatively seizure at the sampling terminal.

Instead of using a needle to aspirate and spray in standard mass spectroscope a prototype system

(Echo-MS) is used as an acoustic mass spec which transmits a sonic signal through liquid producing a "mountain of liquid on the surface". There is use of 384-well assay plate, which directly introduced in Opens Port Probe (OPP) sampling interface. Captured droplets are diluted into a continuous flow of solvent and the OPP liquid stream is drew out to the MS electrode followed by the ionization of sample through electrospray before introducing in MS. ADE transmit these droplets into MS rapidly with high accuracy and precision (Sinclair et al, 2016). Each single well possesses the power to transfer liquid from source well to target well with the feature that the volume may be modified at every transfer making it useful for experiments concerning with doses. Later on, it was also confirmed that the capacity to send liquid from different wells to the same target well build combinatorial screening much simpler (Chan et al, 2016; Cross et al, 2016; Salzer et al, 2016). The acoustic signals can be transmitted to MS with three sample/s in the form of minute droplets using a microtiter plate. Consequently, signals obtained from MS will be very sharp and the presence of ions is recognized within fraction of seconds.

The droplets of about 2.5 nl (nanoliter) are generated from the surface of the liquid and transferred to MS using acoustic signals where, the droplets disintegrate into hundred of minute droplets i.e., fl (femtoliter). The generation of 2.5 nl droplets by ADE system and a technique is described in Figure 2.

During the phenomenon, surface tension also plays a major role. Heavier drops may pull liquid at the surface, creating satellite droplets. Sample solutions having lesser surface tension along with greater droplet size generally tend produces more and larger sized satellites. The key feature is to regulate the out flow of the droplets. Few of the customers were able to produce drops of 50 nanoliters consistently, but definitely in much larger time. However, the customers can't be deviated from the real sense, since this fact can't be feasible with every fluid. Acoustic allotting is mainly appropriate to the field involving very low-volume (up to approx. 1µl). Jeff Lusen, field application specialist at BioSero (Langhorne, PA) explained that an equitable numbers of acoustic dropping discharge occur for extent of volumes between 1-5 microliters, but generally not more than that (DePalma Angelo, 2017).

Attaining larger volumes in minimum time through multiple dispensing is very challenging. Dispensing various drops to attain particular volume takes very less time through the use of acoustic signal which is much lower than the time required to settle liquid drops of DMSO which was basically 120 ms for 10 drops. Squeezing the time between the ejections of droplets is difficult since each drop involves mechanical equilibrium before releasing next droplets. The sample may directly be transferred to mass spectrometer from microtiter plate wells. When the Acoustic signals pass through the analyte, a mist of minute droplets (about femto liter sized droplets) is produced followed by their charging with electric field. This charge is then transferred to the analyte as the droplets abandon in transferring to the mass spectrometer recognizing the molecule on the basis of mass to charge ratio (DePalma et al, 2018)

Advantages:

Below are some advantages of acoustic liquid handling system (Report on ELHT, 2017)-

1. It requires very low amount of sample (1-5 nl).
2. The method is able to generate multiple charged species of the analyte.
3. More compounds can be screened at the same cost.
4. The use of such methods can cut the cost of high throughput screening by about 80%.
5. The system can allow even generating 10,000 data points/hr.
6. Acoustic-MS, with enhanced advantage in targeted drug delivery systems.
7. High precision and accuracy.
8. Touchless and contamination-free, Acoustic Mist Ionization Mass Spectrometry (AMI-MS) can analyze up to three samples per second. As a result, the rich data provided by traditional mass spectrometry can now be applied to dynamic kinetic assays and to large-scale profiling across multiple biological analytes.
9. Acoustic-MS hyphens the pace even with low quantity of samples for analysis.

Acoustic use in mass

Mass spectrometers can quantify the target analyte present in a matrix like drug metabolism studies, kinetic studies, efficacy experiments. Acoustic-MS hyphenation can provide the better insight for identifying the target compound or component rapidly. This technique has established the capacity to considerably decrease the analysis time in targeted drug delivery and other applications. In AMI, the sound plays an important role in injecting liquid samples in the system instead of conventional method of using needle to introduce the same. Here, a fine spray of charged bead is elevated from the top of a liquid sample and reach into the MS system. The acoustics generates approximately 500 bursts of droplets per second, however, for some analyses only ~160 droplet bursts are required for the sample to give adequate ions. The liquid droplets once reached to MS exhibit extremely intense peak and species are recognized in 50 ms of acoustic transducer. Additionally, simple protein and peptide molecules are analyzed through AMI. Data is obtained through a sample system utilizing ADE to transmit minute droplets in a quick, accurate and precise manner straight into the MS (Westphall et al, 2008). Below is the data obtained by ADE to transfer to mass spectrometer by varying number of droplets, injectable volume applied frequency to the transducer. (Figure 3)

Unlike various existing technologies, liquid handling is executed over smooth surface of a programmable chip, where dynamic tracks and different operational blocks along with detecting elements are chemically specified through standard lithographic techniques. The propulsion of the liquid, it’s monitoring and computing the routine elements are established on the basis of electrical excitation mechanical surface acoustic waves, disseminating through the chip surface. Several new instruments have been devised on the basis of this acoustically driven microfluidic technique that include a micro-array hybridization chamber, non-invasive miniature mixers and chip-based PCR reactor, etc (Achim, 2006). Applications

Numerous benefits of touchless transfer have made Echo® liquid handling systems a widely used technique in recent years. It has made remarkable change in analysis time, cost, accuracy, precision, etc for applications in various fields like biotechnology, pharmaceutical service laboratories, drug discovery, drug combination therapy, genomics, cell based assays, diagnostics, RNA interface, reverse transcriptase-PCR assays, personalized medicine, etc (Elkin et al, 2015; Mitchell et al, 2015; Reddy et al, 2015; Olechno, et al, 2016; Cain-Hom, et al, 2016; Agarwal et al, 2016; Nebane et al, 2016; Roberts et al, 2016). In drug discovery the touchless transfer can produce more accurate values of IC50. Next generation sequencing and PCR where larger volumes are utilized through acoustic dispensing is valuable. The advantage of transferring low nano liter volumes of cells, enzymes and other genomic solutions provide with the amended results (Masse et al, 2005; Sileikyte et al, 2010; Bardelle et al, 2015; Bisignano et al, 2015).

CONCLUSION

From the point of analytical view, using smaller volumes and getting higher throughput are the challenges for future advancements. In the technique ADE, the complete touchless transfer method has gained much importance in recent years. Low consumption of volumes of reagents, high speed, cost effective, no contamination, easy identification of unknown compounds in a matrix, high throughput value and several other advantages have made the technique well established in recent years. Recently, *Labcyte* in collaboration with *AstraZeneca* and *Waters* are stepping up for Acoustic Mist Ionization (AMI) as a new revolution.

Fig.1: Echo® 650 LIQUID HANDLER

Fig.2: Reference https://www.youtube.com/watch?v=aBvjhy6MriE

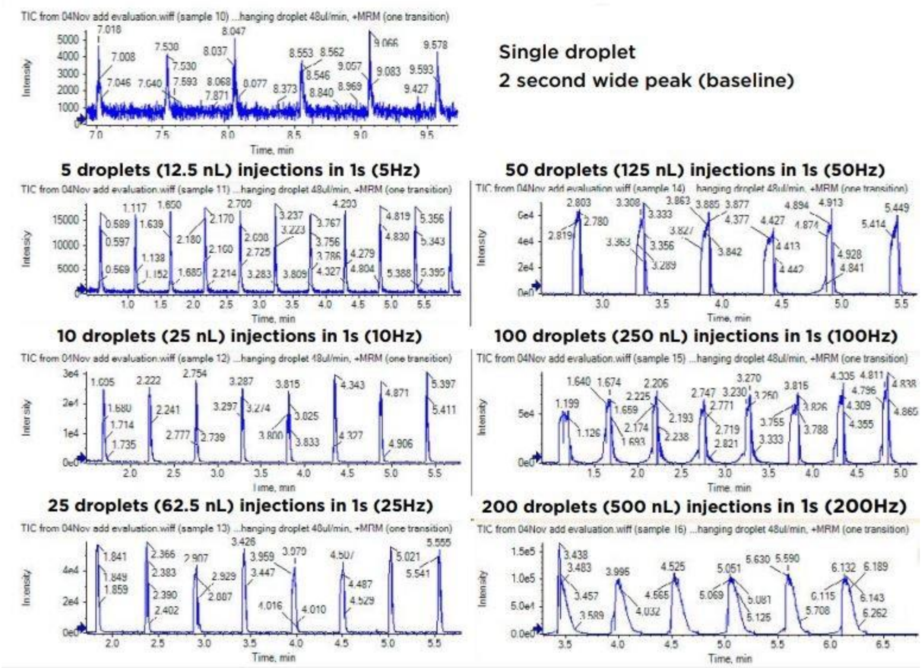

Fig.3: Mass spec data obtained after acoustic droplet ejection (Reference: Lucien Ghislain, Labcyte Inc., San Jose CA, USA Poster Presentation).

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