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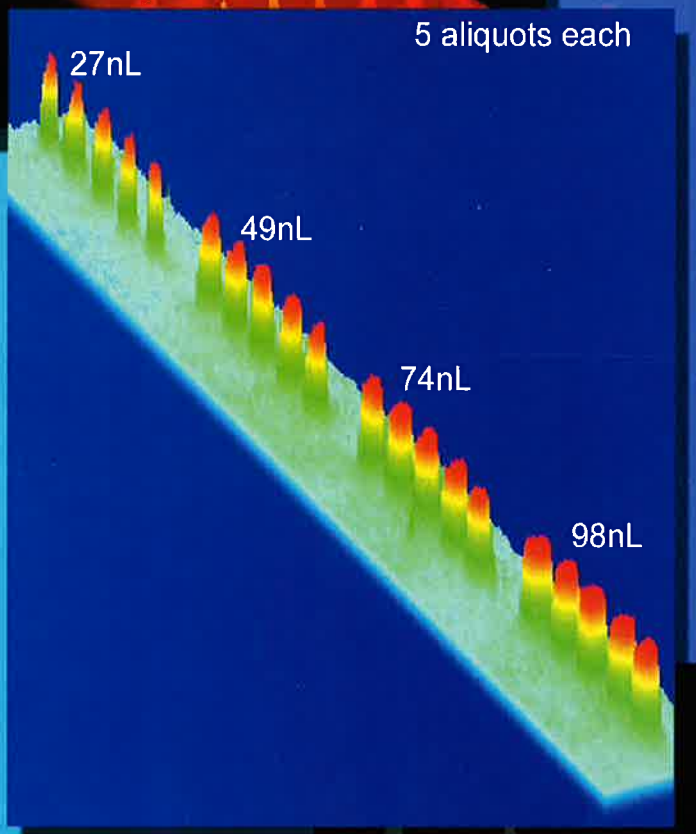

AUTHORS
P. Aggarwal, A. Amirav, K. Chen, S-J. Chen, S. Fantini, M.A. Franceschini, Y. Hoshi, T. Kim, S-H. Nam, A.D. Sauter, Jr., A.D. Sauter, III, M. Tamura

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Nanoliters onto media: Use of electric induction

Andrew D. Sauter, Jr. and Andrew D. Sauter, III

A few years ago while developing models to estimate electrospray ionization (ESI) MS-MS ion current or response, it was recognized by the authors that ESI-based source introduction could yield very accurate and precise flows that might be useful for dispensing and other purposes.[1] They also recognized that ESI has poor properties as it relates to moving liquids, including adverse electrochemistry, and of course the fact that it can spray. Other electrokinetic techniques, such as electrophoresis and electroosmosis, also transport solutions, but have adverse properties as well, such as joule heating. Moreover, the fact that they must be coupled end of reservoir to end of reservoir inhibits or complicates functions like dispensing. In the course of experimenting with ESI, it was postulated whether electric induction might be used to transport liquids. In a manner loosely analogous to magnetic induction creating a flow of electrons in conductors, yielding electricity, the authors wondered if electric induction could be used to assist transport liquids onto media and into receivers. Experiments showed that fields could be employed to this end and a U.S. patent was awarded.[2] Additional patents are pending. The essence of one configuration of the device is shown in *Figure 1*.

Figure 1 shows the general instrument schematic: one configuration, one operational mode with three channels dispensing a liquid to the target; *N* channels are possible. Typically, a voltage (e.g., approx. hundreds to kilovolts) is sent to the inductor, which inductively charges the liquid to higher energy. Upon showing the dispensers to areas of lower energy, flow ensues. The number of dispensing channels can be increased from 1 to 96, but much higher numbers are possible. Figure 1 shows dispensing where there is no contact, but it is also possible to dispense liquids when there is contact between the dispenser and the target. Plus, if the conditions are right, one can also generate a spray as in an ESI interface to MS. Other options can be added to yield very interesting capabilities.

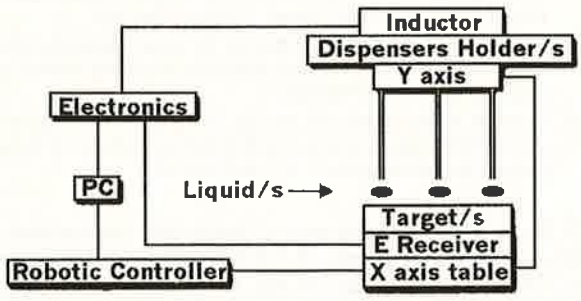

Figure 1 N *channel dispenser (nanoliters, microliters, or both).*

Typically, a positive potential (V+) is applied and cycled (μsec–sec) for time *t* to the inductor. The dispensers and the liquid become immersed in the field and are charged (at higher energy). When the charged liquid is presented to area/s of lower energy, the liquid seeks lower energy and flows to the target, which can be paper, thin-layer chromatography (TLC) plates, microscope slides, or multiple-well plates. Repeating the cycle with movement allows one to place nanoliter and microliter quantities of a liquid or many liquids from *N* channels at a time with one energy source. Such liquid transport has been termed induction-based fluid (IBF) movement. This paper discusses dispensing onto paper media.

Electrokinetic techniques, such as electrophoresis and electroosmosis, also transport solutions, but have adverse properties as well, such as joule heating.

IBF movement (e.g., for dispensing) does not exhibit adverse electrochemistry; since the fluid is not in electrical contact with the soution, there can be no faradic processes as in ESI. Also, the liquid does not have to spray, and only one working circuit is required, unlike jet-based sprays, where each dispenser has its own circuit. Hence, the price of such instrumentation does not have to increase linearly (or faster) with the number of channels. The technical/economic benefits of IBF facilitate parallel operations (e.g., dispensings). Arranged properly, there is virtually no joule heating since the energy is released kinetically. Numerous experiments on video (see Ex-

perimental section) presented at PittCon® 2000 and 2001 showed that fields can be turned on and off rapidly, so that very precise flows could be realized in continuous or discrete mode (e.g., 75 nL ± 3 nL at 1 SD), with liquids held in appropriate containers with scales or otherwise calibrated.

IBF has unique properties. It can cause the movement of different liquids, in different containers, transporting liquids so that multicomponent/parallel solution manufacturing is possible. Also, IBF powers numerous channels simultaneously, in fields of different shapes and functional form; therefore, powerful geometric arrangements are possible that can reduce the costs of dispensing equipment in relation to the often expensive robotic component. Moreover, since fluid movement is required for filtration, solid-phase extraction (SPE), LC, electroblotting, and many other modern manufacturing and laboratory operations, dispensers containing such media have been shown to perform all of these processes. In fact, serial functions have been demonstrated in a highly parallel manner (i.e., executing multiple, different, or same functions one after the other, many units at a time). For example, in one set of experiments, instead of three tubes (as shown in Figure 1), 24 LC columns made from PTFE were used to separate a mixture of food dyes. IBF provides a wide dynamic range of flow rates (µL–pL/sec) and a superset of chip-based flow rates; thus, the device can operate in the macro and micro world. In a number of application areas, as reviewed by the U.S. Army and Navy at an invited presentation at Edgewood Arsenal (Edgewood, MD), the Nanoliter (**Nanoliter**, Henderson, NV) was rated as key emerging technology despite (and perhaps because of) its simplicity.[3]

Using nanoliter quantities of reagents minimizes repeat human exposure to a wide array of chemicals, and waste disposal costs can be reduced.

Nanoliters and induction

Capabilities not withstanding, one must ask the questions: "Why dispense nanoliters?" and "Why use induction?" For dispensing, the use of nanoliters is more economical since less (often expensive) reagents are needed. For example, changing an analytical protocol from a 10-µL dispense to 25 nL can save a factor of 400 in cost, at 100% reagent utilization. (Other applications, such as HPLC, can save very large sums of reagents/expense as well, but only dispensing is being considered here.) Also, using nanoliter quantities of reagents minimizes repeat human exposure to a wide array of chemicals, and waste disposal costs can be reduced.

In answer to the question, "Why use induction?" we can refer to an earlier question posed in *Photonics Spectra*: "How will we handle nanoliters?"[4] Fields have no mass and can be gentle, and provide just the right amount of energy to place a liquid onto media or into a receiver (e.g., an instrument). IBF also offers many capabilities, such as direct dilution of six orders of magnitude without intermediate solutions. If, for example, a beaker containing 100 mL is placed on the receiver and 10 nL is dispensed into that beaker, that volume could be diluted by a factor of one million in one operation. Additionally, if many 1.0-mL vials were placed on the receiver, two or three dispensers were placed above/into each 1.0-mL vial and the inductor were activated, *N* component solution manufacturing could be realized. To date, only preliminary experiments have been conducted along that line. The patent[2] discusses parallel filtration, SPE, LC, and serially parallel derivatives of these functions. This paper is limited to one simple function: parallel nanoliter and microliter dispensing.

Experimental

Each experiment was acquired with the alpha prototype instrument (the Nanoliter) shown in *Figure 2*. It is shown with two-axis movement and a video camera, but other configurations are possible, such as one that uses a Peltier device (**SupercoolUS.com**, San Rafael, CA) to chill nanoliters to create Nanoliter-sicles,[5] which is not addressed herein.

All dispensings utilized IBF with noncontact between the inductor and the target. Dispensers had micrometer-scale orifices. Applied positive dc potentials of hundreds to kilovolts gave currents in the nA–µA range. The pulse durations ranged from milliseconds to seconds on low-millimeter center-to-center dispense geometry. The mechanism of operation is given at nanoliter.com. Snippets of a video can be found at this Web site, along with other pictures and information. A professionally edited ver-

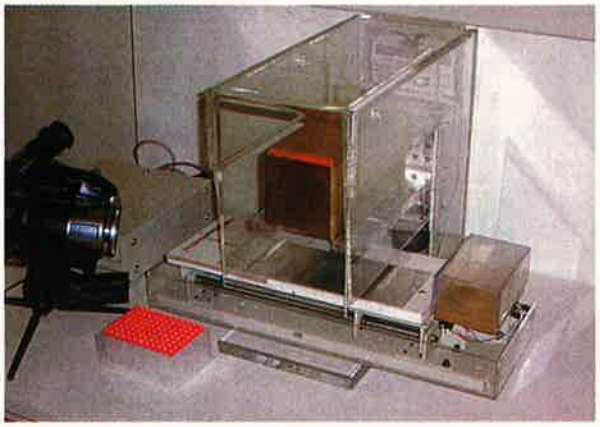

Figure 2 *Alpha prototype of the Nanoliter, shown according to the schematic in Figure 1.*

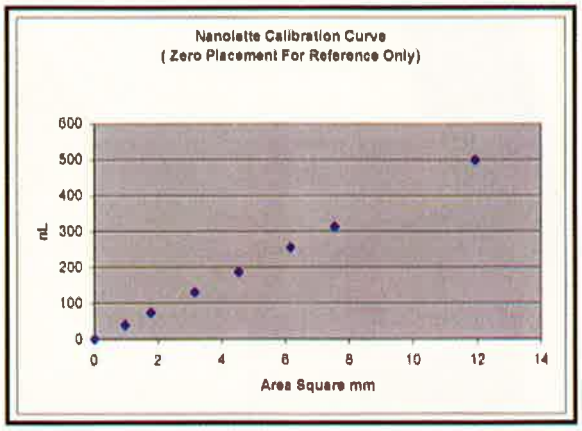

Figure 3 *First calibration curve for area versus dispense time (converted to nanoliters) from the calibration device. The Nanolette is based on the first principles given in the Hagen-Poiseuille equation, but is subject to the assumptions discussed in the text.*

sion has been released showing examples of dispensing numerous nanoliter aliquots using organic liquids, 50% whole human blood, dimethyl sulfoxide (DMSO), hexane, methylene chloride, and other liquids being transported with high accuracy and precision in nanoliter and microliter modes.

The Nanolette is a handheld device (patent pending) for directly dispensing nanoliter volumes of a liquid onto media (**Nanoliter**). The dispense time is varied, creating blots of different size (a calibration curve) onto media. Since the blot area is directly proportional to the volume as, for example, has been done for years in tests such as the dot blot ELISA, the Hagen-Poiseuille equation[6] for laminar flow is presumed to be operative. Then, one or more areas can then be generated using standard devices (e.g., microliter syringes; calibrated capillaries; pipets; or other standard, off-the-shelf devices) to correlate the area with a known volume either manually (*Figure 3* shows the first plot made from a Nanolette) or using the particle analysis option of Vision Builder software (**National Instruments**, Austin, TX). In conjunction with such calibrations, Vision Builder version 6.0 software was employed in a semiautomated mode to locate and identify blots and write area/pixel lists to files. In other applications (such as dispensing nanoliters into vials) alternative devices might be employed to independently calibrate the dispense time axis, from multiple-well plate readers to mass spectrometers using NIST or truly primary standards.

Results and discussion

IBF allows the placement of nanoliter/microliter quantities of liquids onto media (e.g., microscope slides, paper, and nitrocellulose) and into a wide array of vials (multiple-well plates, small 1.0-mL vials, and large holders such as beakers).[1] Applications include the manufacture of products, standards, and other solutions; matrix-assisted laser desorption ionization (MALDI); chemical analysis by IR and other types of spectroscopy; TLC; LC-MS-MS; microhigh-throughput screening; DNA/RNA analysis; clinical applications (e.g., dispensing human blood or other liquids onto microscope slide/s); and forensic applications (saving crucial evidence). This article discusses only the simplest mode of operation of IBF: single- and multiple-level parallel nanoliter/microliter dispensing onto media.

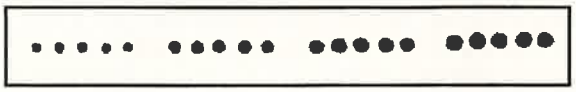

Figure 4 *Five aliquots of a single solution (one channel), 5, 10, 15, and 20 sec per dispense. The figure, a .jpg file, was output from Vision Builder after scanning. The figure is expanded. The true size can be estimated, since the within-group blot-to-blot center distance was approx. 2 mm.*

Single-level, single-solution dispense varying only time

Figure 4 shows the simplest example using a single solution and one dispenser to transport nanoliters onto paper five times at each of four times/levels. From the highest level (largest spots) to the lowest levels, the CVs acquired using the Nanoliter, solution drying, and Vision Builder for detection and integration exported to Excel (**Microsoft**, Redmond, WA) were calculated as 5.4, 6.5, 6.8, and 14.0%, respectively, with estimated mean volumes of 98, 74, 49, and 27 nL. The individual sources of error have not, at this point, been separated. Nevertheless, these data indicate that IBF can attain reasonable precision and accuracy in the lower nanoliter range. In fact, it is asserted that low nanoliter precision and accuracy will routinely approach CVs on the order of 3–5% without great expense using IBF.[2]

Multilevel nanoliter dispensing type 1

Another type of multilevel dispensing is demonstrated for a two-channel system. The two dispensers had different cross-sections. In this mode, the Nanoliter had energy applied at a constant level to the same liquid, but at five different time intervals (0.5, 1.0, 1.5, 2.0, and 2.5 sec) producing five times two, or 10, different dispensing volumes (*Figure 5*).

Multilevel nanoliter dispensing, type 2

A major benefit of IBF is that electric fields can penetrate materials such as vials and paper. Thus, many devices that are not designed to be electrokinetic (e.g., quartz tubes, glass and fused-silica capillaries, glass microliter syringes, and hybrids) can be made suitable for IBF dispensing. Depending on their geometry and other factors, many different experi-

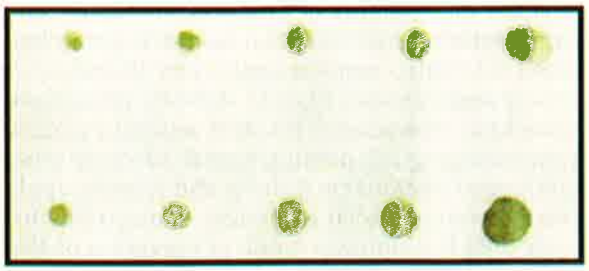

Figure 5 *Blots from a two-channel version of the Nanoliter in which the cross-sections of the two dispensers are different and the same liquid is activated five times at 0.5, 1.0, 1.5, 2.0, and 2.5 sec each. This yields 10 different volumes ranging from approx. 175 to 1310 nL. The reader is welcome to plot the areas of these blots to see if the intercepts approach zero and how the slopes are related.*

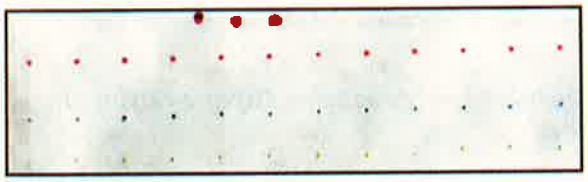

Figure 6 *Three liquids dispensed at three different rates from three different radii, 7 sec per tridispense. Mean values estimated as discussed previously: green, 71 nL, blue, 143 nL; and red, 189 nL, with CVs of 25.6, 19.4, and 11.8% respectively. Note: The source of error involves the entire process of dispensing, drying, and software/data analysis.*

ments can be performed. Variables that can be changed include the number of liquids; the cross-section; the number of channels; and the time, energy, or geometry of the dispenser. *Figure 6* shows the output of the Nanoliter with a three-dispenser head with three different liquids, dispensing at the same time (7 sec per row of three simultaneously). These data visually show how different liquids can be dispensed at different rates at the same time at one energy. With the multilumen advantage (using many tubes), *N* component solution dispensing may be possible using a device that has no moving fluidic parts, as discussed above. The implications of using a version of the Nanoliter in this application is significant since tens or hundreds of *N* component solutions could be made in seconds with one voltage source and one robotic sample manipulator or fraction collector. This should be extremely economical and of major interest to those making standards or drug products. In fact, moving to the nanoliter ranges could offer dramatic reductions in the production cost of medicines because the amount of expensive diluent and/or other components could be greatly reduced. In fact, such technology could impact how expensive chemicals are packaged.

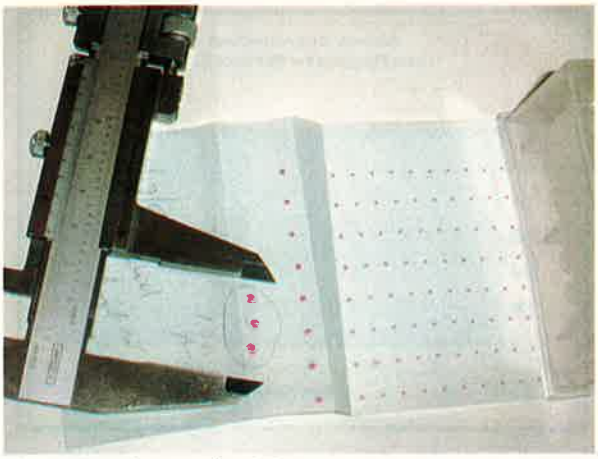

Figure 7 *A dispense for 3.0 sec per each of 10 rows. One dropoff dispense is shown as well. Solvent is a 50% ethanol/water solution containing red food dye FD&C red 40 for visualization.*

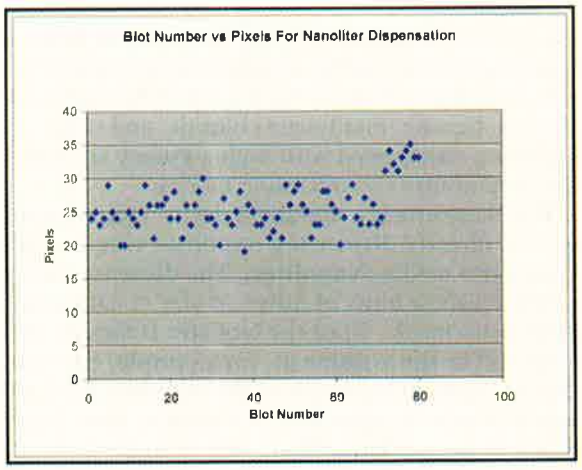

Figure 8 *Data output from Vision Builder. (See nanoliter.com and ni.com for more information on fluidics and machine vision.)*

Parallel nanoliter dispensing and single-level, single-liquid, eight-channel

A digital picture of output from an eight-channel configuration of the Nanoliter is shown in *Figure 7*. These data were acquired in approx. 3 sec per eight-channel row for a total acquisition time of approx. 30 sec. From a related file, Vision Builder was employed to locate and quantify the 80 blots automatically in under 1 sec, yielding the data graphed in *Figure 8*.

In one of the first applications of Vision Builder to older data from the Nanoliter, two groups of data were observed, as shown in Figure 8. For all 80 blots, a mean of 25.5 ± 3.6 pixels for a CV of 14.0% was observed. Eliminating the eight blots at the end of the set gave a mean value of 24.6 ± 2.3 pixels, improving the CV to 9.8% for blots that were previously esti-

mated to have a mean value of approx. 54 nL. (Blots 73–80 are the leftmost blots in matrix 80 in Figure 7.)

Closer visual inspection of these data showed that the first set of blots appeared to be somewhat larger since, in this acquisition mode, the time from the alignment drop to the first drop required an extra distance (i.e.,time) to travel. Hence, in this early experiment, an extra volume was apparently inadvertently dispensed after the dropoff dispense. This was an operator error that is still being considered. That said, Vision Builder revealed a data set flaw in one of its first applications to data that had not been noticed previously. Few things are more valuable than finding the uenxpected. These data are still being investigated for precision and accuracy.

It should be noted that parallel nanoliter dispensing, as performed here, is a rapid process in which errors due to dispensing, drying, calibration assumptions, and software detection and integration are inherent. All things considered, the data indicate that low levels of solvents can be dispensed with reasonable accuracy and precision in parallel in the nanoliter range using the Nanoliter. In fact, single-level CVs on the order of 3–15% are shown in the video with a range of solvents including DMSO, 50% whole human blood, methylene chloride slurries, and hexane. For larger volumes (100–10 µL), precision and accuracy of a few percent have been demonstrated, and picoliter dispensation has also been demonstrated, but it is not addressed herein.

IBF allows one to quickly transport liquids at many different rates and patterns using a range of robotic options and different components to multiple effect.

Evaporation: A potential complication?

Evaporation was characterized by Maxwell 135 years ago, and hence it can be estimated. A 100-µm drop of ethanol has an evaporation rate of approx. 1.7 nL/sec. Therefore, for very small quantities (15–1.0 nL), dispensing should be fast compared to that time scale. Speed is another advantage of IBF. In the referenced video and at nanoliter.com, five 48-channel, 48-solution dispensings of approx. 102 nL ± 15.1% in 12 sec are shown. Dispensings from tens of seconds to msec/µsec give IBF-based dispensings of a wide dynamic range and wide utility for many types of macro and micro functionality.

As a practical matter, the objective of many types of modern dispensing in, for example, the areas of genomics or proteomics is to deliver a known quantity (e.g., femto/picomoles—a volume times concentration) of highly polar reagent to a given locale. Kebarle[7] has shown, in studying desolvation under much more vigorous conditions (i.e., ESI, where the liquid, actually an aerosol, is at temperatures of 200 °C), that polar entities prefer the liquid phase, since the free energy of desolvation of polar molecules is high. Thus, there is not enough energy for polar solutes (e.g., biopolymers) to launch into the gas phase, even if the solvent is evaporating. Therefore, low nanoliter to picoliter dispensing goals may be realized in such systems, even when evaporation occurs. Of course, care is warranted.

Summary

Four simple types of dispensing from the Nanoliter device have been shown in which the number of channels, channel geometry, their connection to one or more liquids, and the energy activation time can be varied to produce different types of output. An example of output from single and multichannel nanoliter dispensing shows that electric fields can help transport liquids and that this patented approach has merit. IBF allows one to quickly transport liquids at many different rates and patterns using a range of robotic options and different components to multiple effect. The technique permits not only the transport of nanoliters and picoliters, but it can do so in many different ways to accomplish a number of goals as it executes a variety of functions when the dispensers are fit with filters, SPE media, LC phases, and other devices.[2] The technology is economical and ecologically sound, since it can reduce the utilization of expensive reagents and minimize waste disposal costs. It reduces unnecessary human exposure in the laboratory and manufacturing processes.

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*Mr. Sauter, Jr., is Founder and President, **Nanoliter,** 217 Garfield Dr., Henderson, NV 89014, U.S.A.; tel.: 702-896-5413; fax: 702-896-5948; e-mail: asauter@nanoliter.com. Mr. Sauter, III, is an electrical engineering student, Santa Clara University, Santa Clara, CA, U.S.A.*

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