Thermal Inkjet System to Enable Picoliter Dispense of Pharmaceutical Compounds

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Abstract

A system designed to dispense small-molecule compounds dissolved in Dimethyl Sulfoxide (DMSO) into ANSI-standard wellplates has been developed by Hewlett Packard (HP). This system, based on HP's Scalable Printing Technology (SPT), includes a disposable TIJ (thermal inkjet) dispensehead cassette designed specifically for the jetting of DMSO. Each cassette uses open 7μL reservoirs that allow a researcher to load fluid directly into the dispenseheads. The system also includes an instrument that drives the dispenseheads and moves wellplates below the fixed cassette. The system finally includes software designed to specify the amount of fluid dispensed into each well, rendering complicated experiments to be simple to both design and execute.

Picoliter-dispense technology is particularly suited for the creation of direct compound titrations in well plates commonly used for dose-response experiments. Advantages over traditional serial dilution techniques include a reduction of carry over and accumulated error, an improvement in precision, and a reduction in compound usage. Additionally, the HP system enables a simpler workflow and makes finely-spaced dosages practical.

This paper describes key features of the HP system and performance data from pharmaceutical beta sites. It also describes the advantages of a TIJ-based compound-dispensing system relative to existing pipette-based or PIJ (piezo inkjet)-based systems. Finally, it describes some of the advantages of using a non-contact Digital Dispenser in this application.

Introduction

Serial dilution is routinely used in pharmaceutical research to create varying concentrations of compounds for dose-response analyses, for secondary screening, and for various absorption, metabolism, toxicity, and compound-compound interaction studies. The comparatively large minimum dispense volumes of existing technologies such as microliter-scale pipetting require a slow and expensive workflow including multiple serial dilution steps to span the concentration range of interest [1].

The solution developed by the Specialty Printing Systems division of Hewlett Packard, relies on the ability of SPT to reliably and rapidly dispense discrete picoliter volumes to create titrations without requiring time-consuming serial dilution steps. SPT directly titrates a solution by dispensing single drops to achieve low concentrations and hundreds or thousands of drops to reach high concentrations. These direct dilutions achieve an independently generated dose for each well without the typical constraints of serial dilution. This digital process easily enables randomized doses, finely spaced doses and dose combinations. These features are impractical with analog processes.

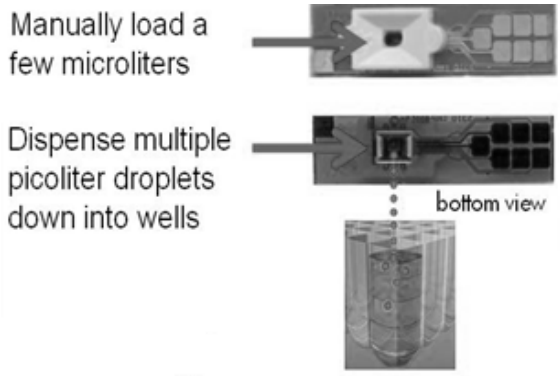

Figure 1: Digital dispensing methodology

Description of System

TIJ dispenseheads have been optimized for jetting DMSO, the standard industry solvent, from an inexpensive, disposable cassette using a benchtop instrument. Each cassette contains eight dispenseheads arrayed at 9mm spacing to match standard multi-channel pipettes. Each dispensehead is topped by a tapered "filling cup" that provides a reservoir for up to 7 μL of fluid. These reservoirs can be filled with as little as 2 μL using a standard pipette and have a dead volume of about 15% of the fill volume, or less than ~1 μL.

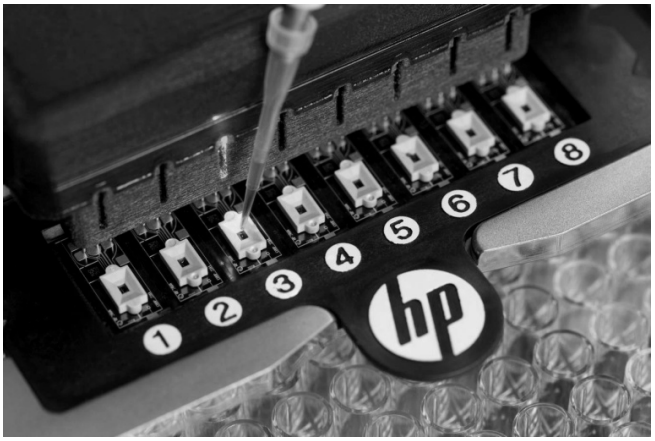

Figure 2: Loading T8 cassette reservoir using standard pipette

HP's SPT platform provides the foundation for this cost-effective titration solution. Each dispensehead is 1.5 mm x 2.3 mm in size, has 22 nozzles, and has a thru-slot that provides a fluidic path for the DMSO to travel from the reservoir on the top of the cassette to the dispensehead nozzles on the bottom of the cassette. The small size of the silicon means that a new dispensehead can

economically be used for each compound, thus eliminating issues of carryover, rinsing, and clogging. The dispensehead operates at 8 kHz per nozzle, utilizing two nozzle sizes that dispense either 20 pL or 14 pL drops with DMSO.

Figure 3: The HP D300 Digital Dispenser

The instrument consists of a pocket to hold a dispensehead cassette, an x-y-z stage that moves a single 96-well or 384-well plate below the cassette, and an automated mechanism that provides electrical contact between the instrument and the eight dispenseheads on the cassette. The instrument also contains an ionizing bar to dissipate electrical charge buildup on the plastic wellplates prior to dispensing drops from the dispensehead. This prevents deflection of the picoliter-scale drops from static charge.

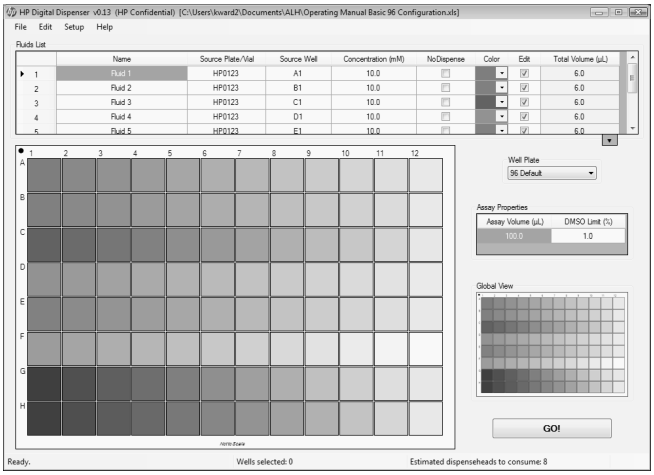

Figure 4: D300 Graphical User Interface

A graphical user interface (GUI) allows the scientist to define the dispense volumes/patterns for an entire plate of titrations before the dispense operation begins. Because the software enables the scientist to enter the assay volume, the stock compound concentration, and the DMSO limit for an assay, the scientist can simply define the concentration endpoints of a titration, the number or wells, the number of replicates, and the titration spacing (linear/log) and the system calculates the required dispense volumes to use in each well. This eliminates errors associated with converting dilution ratios and volumes to concentrations when serial diluting by hand and eliminates the complication of programming pipetting sequences when serial diluting using a robot.

The system automatically determines what combination of large and small nozzles to use to optimize speed and minimize error. Once the dispense operation begins, the GUI prompts the user when and where to load compounds. After the dispense operation is complete, the system generates a report file of compounds, dispense volumes, and concentrations in both tabular and plate-layout formats. These report files can be matched up with plate reader output to generate response curves.

Why TIJ

Thermal Inkjet technology works by using the force generated by gas bubble expansion to drive fluid through a small nozzle. The gas bubble is created by liquid-phase to vapor-phase conversion at the surface of a thin film resistor fabricated on silicon. A common misconception is that this thermal mechanism precludes the use of TIJ in applications that dispense temperature-sensitive materials [2].

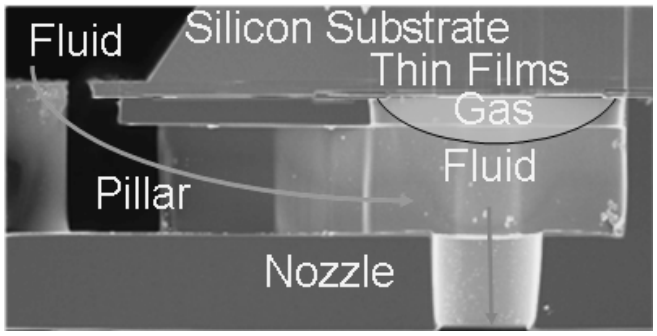

Figure 5: TIJ Drive bubble

The time scale of the heating event on the resistor surface is on the order of 1-3 µs and the vapor bubble completely covers the surface of the resistor within this time. The distance that heat travels in a substance can be approximated by equation (1), where l is the distance that heat travels in time t and α is the thermal diffusivity of the substance (DMSO). Heat travels on the order of 50 nm into the fluid by the time the vapor bubble has initiated. The column of fluid between the resistor and the nozzle is approximately 30,000 nm tall, which means that only a very small fraction of the fluid in the chamber (~0.2%) directly sees the high-temperature event.

$$l \approx \sqrt{\alpha\, t} \tag{1}$$

By comparison, the traditional serial-dilution titration process exposes compound to multiple plastic surfaces that adsorb hydrophobic compounds. The amount of compound lost during traditional serial dilution through adsorptive contact with surfaces of multiple pipette tips and wells is potentially greater than that seen with the digital dispensing process. These losses are cumulative with each successive dilution, and highly diluted compounds (<0.1 µM) are at higher risk of meaningful depletion. Experiments with known compounds at several pharmaceutical

sites have verified that the results from the TIJ process are generally equivalent to those obtained by traditional serial dilution, as shown in Figure 6.

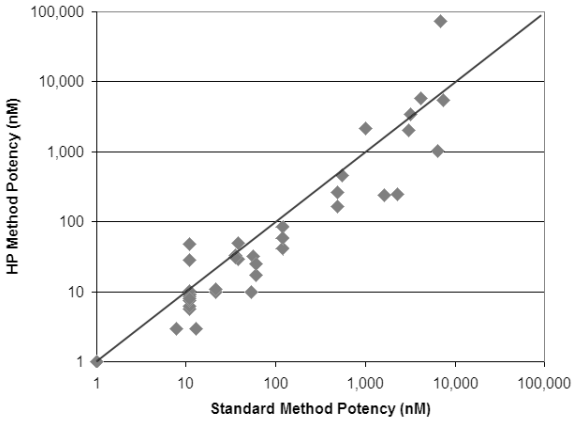

Figure 6: Consistent response seen between HP and standard serial dilution across a variety of compounds and assay types.

Building up doses using picoliter-scale inkjet drops provides advantages in the precision of dispense volumes on the picoliter, nanoliter, and microliter scale. Figure 7 shows the "coefficient of variation" (CV), a volume-normalized dispense standard deviation, for volumes ranging from 14 pL to 500 nL using the Digital Dispenser. CVs of less than 10% are generally considered to be good. While the Digital Dispenser achieves this level of performance for dispense volumes above 100 pL, analog pipetting typically cannot achieve this with single transfers until dispensing volumes are above 1 μL.

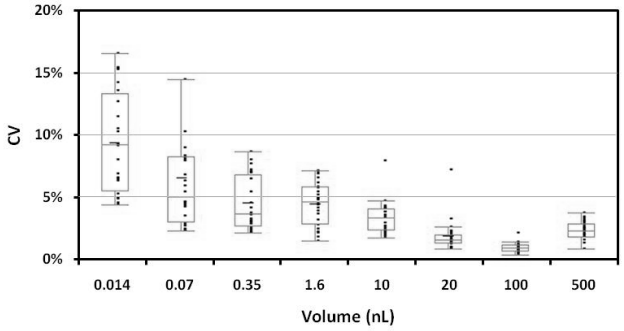

Figure 7: Coefficient of variation with Digital Dispenser using 24 dispenseheads per volume with 10 replicates per dispensehead

TIJ enjoys several advantages relative to PIJ in this application. First, the small firing chamber of TIJ relative to PIJ enables a gradual decrease in the capillary length scale as the fluid moves from the reservoir to the nozzles, which enables self-priming without spitting, wiping, or suction. No fluid is used in preparing the printhead for dispensing and very little dead volume fluid (<1 μL) is trapped in the fluidic channels and reservoir at the end of life. Secondly, the nozzle-packing density of TIJ enables more nozzles to simultaneously jet into a well, even in higher-density (1536 well) plate formats with smaller wells. Thirdly, TIJ is more tolerant of very low-backpressure operation, which enables a simple low-dead-volume fluid reservoir path without foam or active backpressure. Finally, the relative complexity of PIJ systems makes it more challenging to make a cost-effective disposable printhead.

The Digital Advantage

Just as digital printing technology is transforming the traditional analog printing industry by giving users capability that was not practical with analog technologies, digital dispensing provides many advantages over traditional analog methods.

Randomization in Layout

Traditional serial dilution is an analog dispensing process that limits how experiments can be designed. For example, titrations are almost always designed to be spatially systematic and continuous to reduce the likelihood of manual pipetting error or reduce the complexity of robotic-pipetting. Because many assays are sensitive to edge effects in which wells near the edge of a plate react differently from wells near the center of the plate, the mapping of spatially continuous titrations onto these plates can lead to experimental error.

	1	2	3	4	5	6	7	8	9	10	11	12
A	5623	56.2	3162	3162	3162	4642	215	10	5623	56.2		
B	3162	31.6	1000	1000	1000	3162	147	6.81	3162	31.6		
C	1778	17.8	316	316	316	2154	100	4.64	1778	17.8		
D	1000	10	100	100	100	1468	68.1	3.16	1000	10		
E	562	5.62	31.6	31.6	31.6	1000	46.4	2.16	562	5.62		
F	316	3.16	10	10	10	681	31.6	1.47	316	3.16		
G	178	1.78	3.16	3.16	3.16	464	21.5	1	178	1.78		
H	100	1	1	1	1	316	14.7	0.68	100	1		

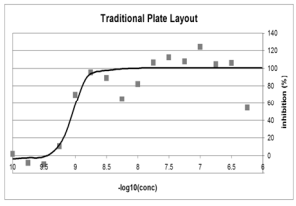

Figure 8: Non Randomized Layout and data

Figure 8 shows a plate layout and the accompanying effective-concentration data and curve fit for the typical spatially-continuous 16-point titration located in the first two columns of the plate. In this assay, the edge wells (A1, A2, H1, H2) have a lower inhibition than they would if these same concentrations were located in center wells. Because of the systematic spatial pattern of concentrations associated with analog serial dilution, curve fitting to these data can lead to bias in the fit.

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	4642	1778	1000	2	7	1	32	3162	3		
B	1	10	3	32	316	32	1000	3162	1	5		
C	6	10	100	316	18	215	15	56	5623	10		
D	316	18	10	3162	2	147	10	32	464	5623		
E	100	1	1000	32	2	1	3	1000	3	3162		
F	10	316	3162	2154	316	22	178	316	3	68		
G	100	3162	1000	100	178	562	681	100	32	1468		
H	46	6	1	1	56	1000	3	562	1	1778		

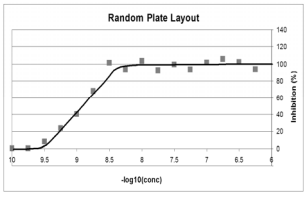

Figure 9: Randomized Layout and data

The original plate layout of Figure 8 was randomized via the system GUI to create the randomized plate layout shown in Figure 9. The effective-concentration data and curve fit for the same 16-point titration found in Figure 8 (now randomized) are also shown in Figure 9. While edge wells still behave differently from center wells, the elimination of the systematic spatial pattern of concentrations results in a reduction of curve-fit bias. While the plate layout shown in Figure 9 would be difficult or impractical with analog pipette-based serial dilution, it is simple with a Digital Dispenser in which every well dispensed is completely independent of every other well.

Co-titration

Co-titration experiments are those in which more than one compound is dispensed into each well to look for synergistic or antagonistic effects. Typically, these experiments are designed with titration replicates of one compound in one spatial direction (left-to-right) on the plate, with titrations replicates of a second compound arranged in the orthogonal direction (top-to-bottom).

Because every well has a unique combination of the two compounds in question, these experiments are difficult to execute with a contact-dispensing analog system such as pipetting. However, these experiments are easy to design and execute with a non-contact Digital Dispenser. One dispensehead is used for each compound and the two dispense patterns are jetted sequentially and independently of one another. This technique can be used for more than two compounds per well. Other dispense operations, such as DMSO normalization can be conducted with additional dispenseheads.

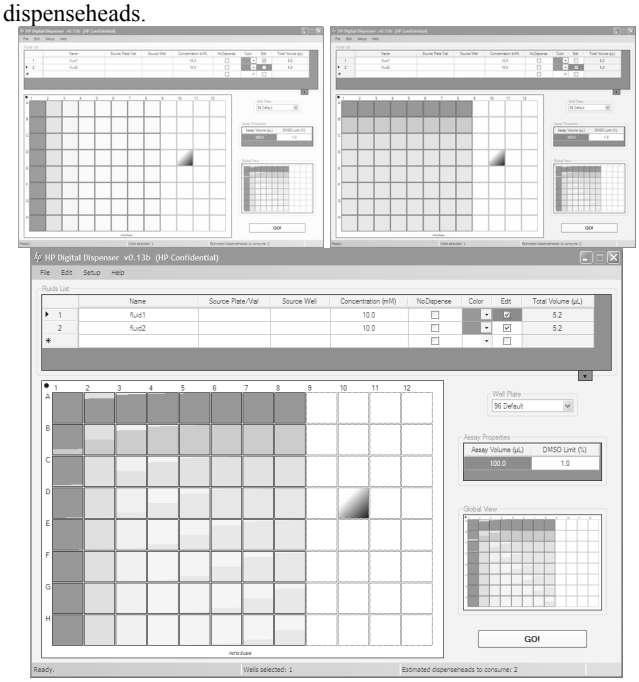

Figure 10: Compound co-titration experiment with two compounds. Compound 1 is upper left; Compound 2 is upper right; Combination of 1 and 2 is bottom.

Finely-spaced doses

With analog dispensing systems, each dilution level requires an additional set of dispensing processes. To increase confidence in data, dilution-levels are often replicated in triplicate as a way to average out some of the noise in the system. A typical titration would be an 8-point titration (8 concentrations) in triplicate, using a total of 24 wells. Creating a 24-point singlet titration instead of an 8-point triplicate requires three times as many serial-dilution steps and three times as much accumulative error in an analog system. However, on a Digital Dispenser where every well is independently dispensed, the time required to dispense a 24-point singlet titration is nearly equal to the time required to dispense an 8-point triplicate.

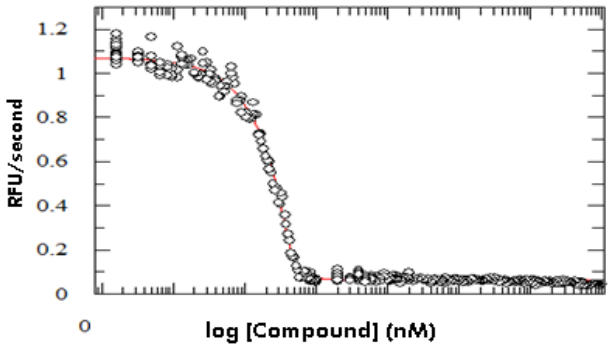

Figure 11: 384-point titration response curve

Using finely-spaced singlets allows datapoints to be better distributed around the response curve. Figure 11 shows this concept taken to the extreme with a 384-point titration. A robotic pipette system requiring 30-seconds per dilution step would take more than three hours to do such a titration and would generate an enormous amount of waste and accumulative error with the sum of the dilution steps. By contrast, this titration requires only a few minutes on the Digital Dispenser and eliminates or reduces the waste of the intermediate dilution plates, fluids, and pipette tips. Digital dispensing allows researchers to obtain more data in the region of interest.

Conclusion

The HP D300 Digital Dispenser brings the advantages of a non-contact digital dispensing technology to the creation of dose-response experiments. The small SPT dispensehead is the key to making a cost-effective disposable titration solution. Experimental layouts that are not practical with analog technologies, such as randomized doses, dose combinations, and very finely spaced doses are now possible and practical.

Acknowledgements

Data for Figures 6, 8, and 9 courtesy of Rob Jordan and Robbie Allen of SIGA Technologies. Figure 11 courtesy of Dan Thomas of GSK UK.

References

[1] Ken Ward et al, Picoliter Dispense to Eliminate Serial Dilution, Miptec (2010)

[2] Jeff Nielsen, Thermal Ink Jet System to Enable Non-Traditional Applications, Proc. IS&T Digital Fabrication Conference, pp 62 (2006)

Author Biography

Jeff Nielsen is a Senior Engineer at Hewlett Packard who has focused on thermal inkjet printhead technology and applications since joining the company in 1993. He is the supplies engineering lead and fluidics architect of HP's Digital Dispensing system. Jeff earned his Bachelors and Masters Degrees in Mechanical Engineering from the Massachusetts Institute of Technology in 1992 and 1993.