

Substrate Dependency of Low Volume Non-contact Dispensing

Introduction

In order to investigate the effects of target substrate on the dispensing of a non-contact liquid handler, high speed photography of dispensing events were taken with varying substrate material and conditions as well as reagent composition. These photographs were compared to the multi-droplet dispensing characteristics under similar conditions with similar target/reagent combinations to observe whether the droplets remain discrete droplets or whether the droplets run together (different applications would require different liquid morphologies). Conditions investigated include:

- ▶ Hydrophobic/hydrophilic surfaces
- ▶ Roughened/static charged surfaces
- ▶ Hydrophobic/hydrophilic reagents

Information may be drawn from this data to guide future designs of substrates intended for use with non-contact dispensing liquid handling platforms.

Background

One of the greatest advantages of low-volume non-contact dispensing is that the dispensing needle or nozzle does not come into contact with the substrate and therefore, dispensing quality is independent of substrate. While this is generally true, as non-contact dispensing moves into more non-standard applications such as medical diagnostics, and established applications such as genomics begin to utilize non-standard materials for use as substrates, increased attention is being paid to the effect that substrate materials and topical features have on the morphology of the dispensed of liquid. There are a wide variety of material properties that have the potential to affect drops when dispensed from non-contact liquid handlers that do not come into contact with the substrate. The most obvious examples are hydrophobicity/hydrophilicity, electrical conductivity, and flexibility of the substrate. There are also a wide variety of desired outcomes for these dispensing events. Often, in the manufacturing of medical devices or biosensors, unusual substrates are used, requiring the reagent to be placed in a very specific position within the features of the substrate. It is often the case that very closely spaced discrete droplets are needed for an application, however, it is also not uncommon for a droplet to be required to fill a designated area of a substrate



Picture 1 — Innovadyne Nanodrop non-contact low-volume liquid handler (IDEX health and Science, LLC)

and not remain spherical. For either desired spatial outcome, attention to the effect that surface characteristics have on reagent dispensing is critical. While it is well known that traditional liquid handlers requiring 'touch off' or 'near touch off' techniques are greatly influenced by the condition of the substrate, non-contact liquid handlers, while eliminating the need to touch the substrate, are still subject to substrate/reagent interaction and to the laws of physics. To illustrate some of these effects we will present data from high speed and standard photographic techniques showing the effect of different reagent/surface characteristics on the behavior of a dispensing droplet.

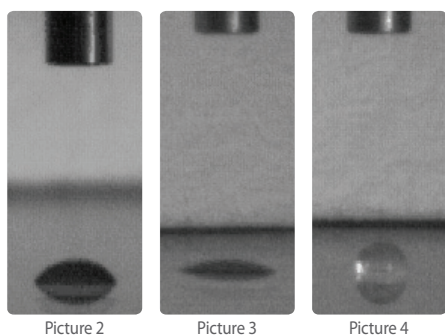
Experimental

The non-contact liquid handler employed is the Innovadyne Nanodrop (IDEX Health and Science, LLC) (picture 1). The high speed camera used for close-up photos of the dispensing events was a Motion Scope PCI 8000 S from Redlake Imaging, which took pictures at a rate of 1000 frames/s. All high speed photos were taken with the instrument dispensing 200 nL at 8 psi (a pulse width of 6965 μ s). For still photos, a Canon PowerShot A610 (5 MegaPixels) was used. For these photos five 100 nL droplets (a pulse width of 3470 μ s) were dispensed with a spacing of 1.5 mm between droplets. A 7901 ionizing bar from Exair Corporation was used to discharge static electricity on Teflon® substrates.

Results/Discussion

Hydrophobicity/Hydrophilicity

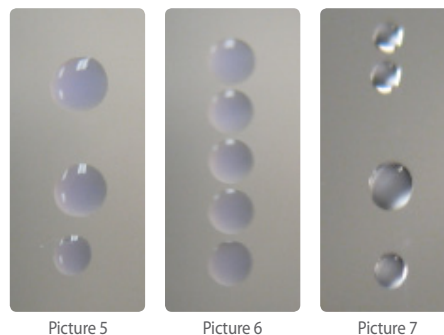
It is well known that water-based materials are attracted to hydrophilic surfaces and not to hydrophobic ones. For non-contact dispensing, this translates into the roundness of the resulting droplet, which can be quantified by measuring its contact angle (the angle at which the droplet makes contact with the surface). For a very highly hydrophobic surface, the contact angle will be very near 90°. For hydrophilic surfaces, the contact angle will be near 0° and the droplet will effectively spread over the target surface. By way of illustration, picture 2, shows a water droplet dispensed onto a moderately clean glass surface. The resulting droplet neither spreads nor does it have a sharp 90° angle. Etching the surface with concentrated HCl overnight causes the surface to become more hydrophilic by virtue of the exposed surface active sites. The resulting droplet is considerably flatter (see picture 3) and spreads over a larger surface area. Conversely, treatment of the surface with a hydrophobic solution (in this case Rain-X™), causes the droplets to form very nice droplets with large contact angles (see picture 4).



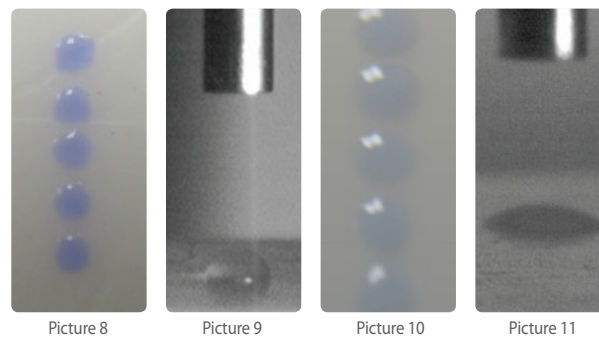
Pictures 2-4 — A water droplet on an untreated glass surface, the same glass that underwent an overnight 14.5% HCl etch, and again after treatment with the hydrophobic Rain-X. All droplets are 200 nL.

Translating this droplet morphology information into multi-droplet formations on the same substrates, picture 5 shows the results of a five drop dispense pattern of water (actually bromophenol blue dye in a tris buffer solution) on the untreated glass surface, while picture 6 shows the same dispensing conditions, but with a glass surface treated with Rain-X. While the untreated surface caused drops 1&2 and 3&4 to merge, the Rain-X treated surface maintained discrete drop formation. It is not surprising that if discrete droplets are the desired result of a dispensing pattern, that the hydrophobically treated surface would be far superior. Conversely, if the reagent needs to be spread throughout an active surface, a more hydrophilic surface is necessary. If, however, the dispensing reagent has a hydrophobic component to it, such as 70% isopropyl

alcohol, treatment of the glass with hydrophobic material is not as helpful (see picture 7). Dispensing onto a strongly hydrophobic target surface such as Teflon®, a water-based reagent results in very good droplet formation (see pictures 8 and 9), less hydrophilic reagents, such as 70% IPA, however, will spread a little bit more (see pictures 10 and 11).



Pictures 5-7 — A set of 5 water droplets on an untreated glass surface (4 droplets are merged into 2) and after treatment with the hydrophobic Rain-X. The same Rain-X treated glass slide with Isopropyl alcohol droplets (two of the central droplets have merged). All droplets are 100 nL with a spacing of 1.5 mm.

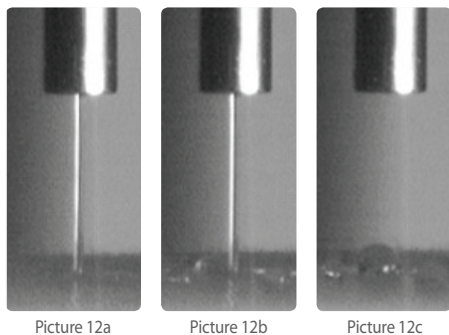


Pictures 8 and 9 — Water droplet formations on a Teflon surface.
Pictures 10 and 11 — IPA droplets on a Teflon surface.

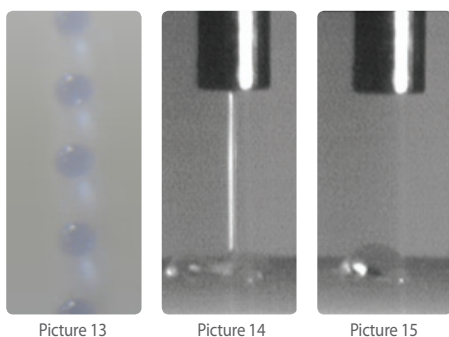
Roughness and Electrostatic Effects

Hydrophilic surfaces, however, also tend to hold onto charge in the form of static electricity. To test these effects, a Teflon surface was roughened with sandpaper. The resulting surface was moderately rougher, but also contained patches of hydrophilic charged surfaces. As a result, the dispensing droplet, while dispensing nicely, once the liquid reaches the modified surface it is immediately attracted to the charged surfaces regions and spreads across the target forming multiple smaller droplets (see pictures 12a-c). After running an ionizing bar over the Teflon surface, electrostatic effects are reduced, resulting in a nicer droplet morphology (see pictures 13-15).

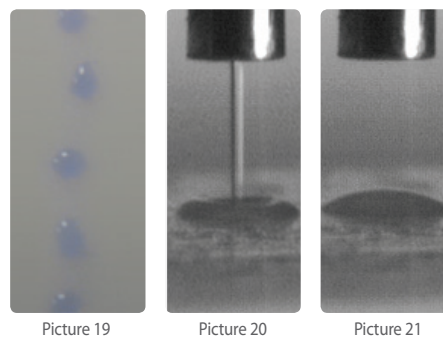
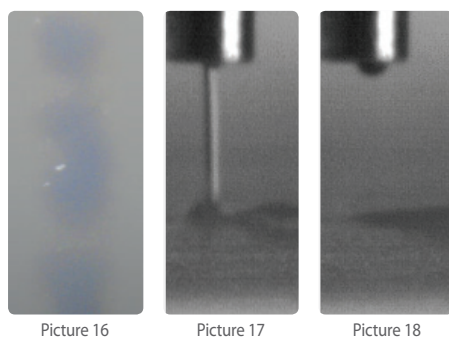
When sanded Teflon substrates are dispensed onto with 70% isopropyl alcohol, which is less hydrophilic but also has less surface tension, droplets are more prone to spreading and even more susceptible to electrostatic effects (see pictures 16-21).



Pictures 12a-c — A single dispensing event with photographs taken at the beginning, middle, and end of the dispense of water on newly sanded Teflon®



Pictures 13-15 — Water droplet formation on roughened Teflon after exposing it to an ionizing bar



Pictures 16-21 — IPA droplets (70%) on freshly sanded Teflon before and after exposure to an ionizing bar. The top row (Pictures 16-18) shows high speed photography of a dispensing droplet onto freshly sanded Teflon before exposure to the ionizing bar. Note the droplet being drawn out from the tip of the dispensing nozzle even when the instrument is not dispensing, as well as the formation of the droplet in an area not directly under the nozzle. The bottom row (Pictures 19-21) shows the dispensing results on a freshly sanded Teflon substrate subjected to an ionizing bar. Note that the resulting droplet is more cohesive than prior to the ionizing bar, and similar to the morphology of the droplet prior to surface roughening. Dispense placement, however, is still not always accurate in this case either due to the rougher surface, or to incomplete elimination of the static charge.

Conclusions

We have shown that the droplet formations from a non-contact liquid handler can be significantly affected by the physical and chemical characteristics of both reagent and substrate. When dispensing a hydrophilic reagent onto a hydrophobic surface, discrete droplets are more likely to result, while droplet spreading will occur if it is dispensed onto hydrophilic surfaces. While this is not a surprising result, it is interesting to note that, as drop sizes decrease, reagent interactions with the substrate surface is increasingly important due to the greater surface/volume ratio of the liquid. Furthermore, the effects of pockets of electrostatic charge can also have a significant effect on the drop morphology and positional accuracy of a non-contact dispenser. While these effects are not likely to be seen at large volumes or in most multi-well microtiter plates, they can have a larger impact for low volume dispensing onto non standard or higher density substrates. In light of this, when designing a commercial system that requires liquid handling as an integral part of its manufacturing or testing process, it is not enough to simply use a convenient material in a convenient topical configuration. There must be attention paid to the treatment of and the interaction of the substrate material with the reagents with an eye toward utilizing surface modifications or hydrophobic/hydrophilic combinations to guide the morphology of the resulting droplet. This attention to substrate-reagent interaction can more consistently achieve the necessary reactions while avoiding contamination of neighboring regions of the substrate.

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