White Paper

RAININ
Pipetting 360°

Positive Displacement Pipetting

Three Recent Examples

Positive displacement pipettes are minor, yet important players in the laboratory liquid handling toolbox.

Positive displacement pipettes are available in two varieties – single aspiration/dispense pipettes, and repeater pipettes. For the sake of simplicity, this paper will focus on single aspiration/dispense pipettes. We will review three recently published papers where positive displacement pipettes played a critical role. These serve as good examples on how these pipettes can be used.

Figure 1: Rainin Pos-D positive displacement pipette

METTLER TOLEDO

1 Applications of Positive Displacement Pipettes

Positive displacement pipettes are used for a number of applications that cannot be accomplished by air displacement systems. For instance, positive displacement pipettes can be used to pipette volatile solutions, which would normally leak out of a tip with an air displacement system. Another application is the pipetting of non-aqueous viscous solutions like glycerol, and aqueous viscous solutions like carboxymethyl cellulose dissolved in buffer. Positive displacement pipettes can also be used to handle viscous biological samples like semen, blood, and saliva easily. For all viscous materials, positive displacement pipettes offer accurate and precise liquid handling versus air-displacement pipettes for two main reasons. Firstly, positive displacement pipettes do not have an air-gap between the pipetting piston and the sample, rather, the sample directly contacts the disposable piston within the pipette tip. The elimination of the pipetting air-gap prevents the expansion of air within the pipetting system during aspiration of dense solutions (i.e. glycerol) which in the case of air-displacement pipettes can lead to incomplete, slow, and inaccurate uptake of liquids into the pipette tip.

Also, due to the piston plunger being within the pipette tip, all of the sample is expelled from the tip upon dispensing – no liquids can stick and be retained within the tip because the plunger makes flush contact with the interior of the pipette tip. A final application for positive displacement pipettes is the liquid handling of standard aqueous solutions. These pipettes are fully capable of pipetting common aqueous solutions, though may only find application when pipetting at non-ambient temperatures where regular air displacement pipettes are not calibrated to function. In summary, positive displacement pipettes excel for liquid handling under the following circumstances:

- Liquid handling of volatile liquids such as acetonitrile, methanol, ethanol, etc
- Liquid handling of non-aqueous viscous liquids, like glycerol, etc.
- Liquid handling of aqueous or biological viscous liquids, like diluted glycerol, pure blood, semen, etc.
- Liquid handling under non-ideal conditions, i.e. in a 4°C cold room, or a 37°C warm room, or in environments where the pressure deviates significantly from standard temperature and pressure conditions.

2 Construction of Positive Displacement Pipettes

The physical construction of positive displacement pipettes confers the pipettes' unique liquid handling performance versus air-displacement pipettes. While the handle of positive dis placement pipettes is similar compared to air displacement pipettes, the liquid end of positive displacement pipettes is unique (Figure 2). The liquid end of positive displacement pipette contains a collet that, when the plunger is fully depressed, can affix onto a pipette tip that contains a piston plunger within a tip capillary. Once the capillary and piston plunger assembly has been affixed onto the end of the pipette, the assembly can then be used for liquid handling. After aspirating and dispensing a solution, the tip can be reused for pipetting until the user decides to dispose of the tip by depressing the plunger past the first stop. This will cause the collet to release the tip and the tip will fall off of the pipette, preferably into a waste or recyclables receptacle.

Figure 2: Positive displacement liquid end with capillary/piston attached

3 Three Case Studies

Numerous examples of the use of positive displacement pipettes have been observed in the literature. We will examine three different publications where positive displacement pipettes have been used to pipette volatile, viscous, or biological samples.

1. Pipetting volatile liquids in GC/MS studies of pea cultures

In the journal Food Chemistry Azarnia and coworkers published a paper titled "Volatile flavor profile changes in selected field pea cultivars as affected by crop year and processing" [1] (Figure 3). In this study, the authors were interested in how differences in field pea cultivar, processing, and crop year affected the volatile compound content of selected field peas grown in Canada. For this work, the authors relied on headspace solid-phase microextraction in combination with gas chromatography / mass spectrometry (GC/MS) to compare the flavor profile of different pea cultivars and evaluate the effect of processing and crop year on the volatile flavor compounds in the peas. For standards, the authors purchased a number of volatile compounds from commercial vendors to serve as standards for their GC/MS technique. For the proper preparation of their standards, the authors pipetted 10 µL of volatile standards using a positive displacement pipette into 10 mL of methanol. After this, 1 mL of the resulting solution was pipetted into 9 mL of methanol and 10 µL aliquots were taken with the positive displacement pipettes for individual or group dilution into ddH2O, or 6 M NaCl. These samples were then analyzed using the GC/MS technique mentioned above. It appears that the authors found significant utility in pipetting volatile mixtures using positive displacement pipettes.

Figure 3: Field Pea

[1)] Azarnia, Sorayya, Joyce I. Boye, Tom Warkentin, Linda Malcolmson, Hassan Sabik, and Anne Sophie Bellido. "Volatile Flavour Profile Changes in Selected Field Pea Cultivars as Affected by Crop Year and Processing." Food Chemistry 124, no. 1 (2011): 326-35. doi:10.1016/j.foodchem.2010.06.041.

2. Pipetting viscous liquids to study cardiac protein oxidation

In the journal Free Radical Biology and Medicine, Snook and colleagues published a paper titled "Peroxynitrite inhibits myofibrillar protein function in an in vitro assay of motility"[2] (Figure 4). In this study, the authors were studying the effects of the chemical oxidant peroxynitrite on cardiac myosin, actin, and thin filaments. To carry this out, they exposed cardiac myosin, thin filaments, and actin filaments to peroxynitrite and would then measure their motility behavior in a microscope assay were the species were observed over time to measure how far they could propel themselves over a defined amount of time. The buffer used in this microscope assay contained 0.5 % (w/v) methylcellulose, which the authors mentioned was very viscous. For this reason, the authors used positive displacement pipettes to dispense the liquid and to prepare new experimental samples. In this situation, the authors found positive displacement pipettes useful for the handling of a viscous liquid.

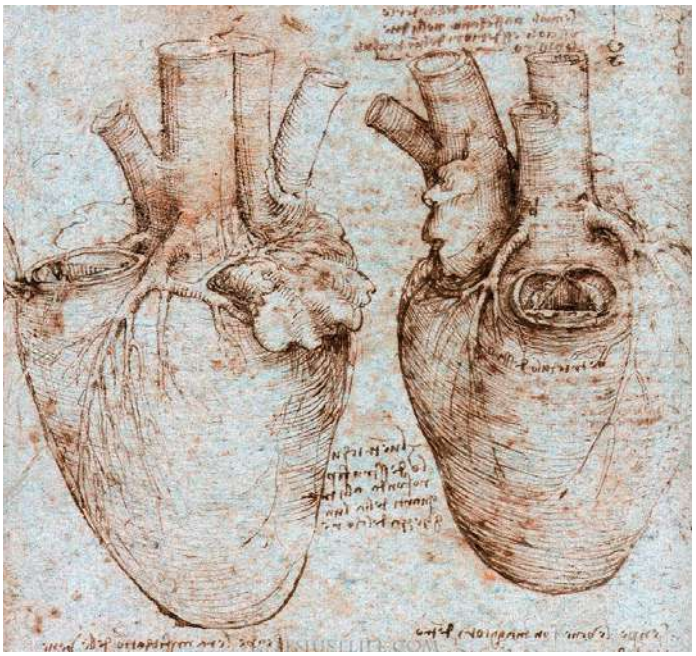

Figure 4: Heart drawing by Leonardo da Vinci

[2] Snook, Jeremy H., Jiahui Li, Brian P. Helmke, and William H. Guilford. "Peroxynitrite Inhibits Myofibrillar Protein Function in an in Vitro Assay of Motility." Free Radical Biology and Medicine 44, no. 1 (2008): 14-23. doi:10.1016/j.freeradbiomed.2007.09.004.

3. Pipetting bodily fluids to measure rooster sperm production

In the journal Poultry Science, Froman and Rhoads published a paper titled "Validation of a spectrophotometer-based method for estimating daily sperm production and deferent duct transit"[3] (Figure 5). In this study, the authors were interested in validating a new method for estimating daily sperm production by roosters. Also, as this method was to be used to evaluate testis output and deferent duct throughput in roosters, the authors evaluated the analytical approach in subsequent experiments. In this study, the authors used a 25 µL positive displacement pipette to add 10 µL of diluted rooster semen samples to spectrophotometer cuvettes containing 2 mL of 3 % sodium chloride solution. After measuring absorbance at 550 nm, a rooster semen standard curve could be generated. Also, positive displacement pipettes were used to estimate the extra-gonadal sperm reserve of roosters. Here, the authors used positive displacement pipettes to measure the volume of extra-gonadal sperm that was forced out of the deferent duct prior to measuring the sperm concentration of this liquid. The total sperm amount was estimated by multiplying the volume of the semen by the determined sperm concentration.

Figure 5: Rooster

4 Conclusion

The studies above show that scientific researchers have liquid handling applications that cannot always be addressed by standard air displacement pipettes. Single aspiration and dispense positive displacement pipettes are recommended for low-throughput liquid handling of aqueous and non-aqueous volatile and viscous solutions. They are also recommended for viscous biological solutions like blood, semen, saliva, etc. The real-life applications and studies referenced above show the performance of positive displacement pipettes can be used to further scientific research. Though uncommon in laboratory liquid handling, the increased use of these pipettes when pipetting under non-ideal, non-aqueous situations might lead to more accurate conclusions and higher-quality scientific work.

3) Froman, D. P., and D. D. Rhoads. "Validation of a Spectrophotometer-based Method for Estimating Daily Sperm Production and Deferent Duct Transit." Poultry Science 91, no. 10 (2012): 2621-627. doi:10.3382/ps.2012-02289.

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