Improve pipetting results in pharmaceutical formulation by using MICROMAN E

Application Note LT805005

Nadège Belhadj, Gilson, France

nbelhadj@gilson.com

In pharmaceutical formulation, properties of used liquids differ significantly from water and are mainly viscous causing difficulties to pipette and residual carry-over. Indeed, problem liquids like glycerol stay in the tip when dispensing. Comparisons between an air displacement pipette and a positive displacement pipette, MICROMAN E, show that more accurate and precise dispensing of viscous liquids is achieved. Thus, the MICROMAN E, in combination with Capillary Pistons, is capable of pipetting and dispensing viscous liquids and water with similar precision and accuracy.

Introduction

Liquid drugs formulation involves pipetting of pH adjustment/buffering agents, tonicity modifiers, viscosity enhancers/reducers, chelating agents and adjuvants. Most of these liquids are viscous and interact directly with pipettes when transferring microliters of solution during the preparation.

Dispensing systems in the laboratory can function either by employing the air displacement principle, or they constitute a positive displacement system. In an air displacement pipette, an air cushion separates the liquid in the plastic tip from the piston inside the pipette. Like any gas, the air cushion between the piston and liquids interacts according to the characteristics of liquids (dense, viscous, surfactant, volatile) as well as partly by lab or protocol condition (temperature variation, humidity). A liquid with a high viscosity flows very slowly into and out of the tip. The use of viscous liquids stays in the tip when dispensing causing residual carry-over. Additionally, if the tip is withdrawn too soon from the liquid reservoir, an air bubble is also drawn into the tip, reducing the liquid volume leading to imprecisions.

With positive displacement pipettes, the liquid does not come into contact with the pipette. The piston scrubs on the inside wall of Capillary Pistons and can dispense the viscous liquids. There is no air cushion, in this way, the physical properties of the liquid have very little influence on the volume of the liquid to be aspirated or dispensed.

This application note demonstrates the advantages of using a positive displacement pipette like MICROMAN® E with Capillary Piston, to improve efficiency pipetting viscous solutions in pharmaceutical formulation by comparison with air displacement pipettes and tips.

Materials & Methods

The results deal with the impacts of characteristic of solutions (viscous) and the type of used pipetting modes (forward and reverse) with an air displacement pipette versus a positive displacement pipette on accuracy and precision of volume gravimetric results.

Solutions with glycerol 100% (1.26g/mL) served as sample material to show the impact of viscosity while pipetting pharmaceutical solutions. Glycerol is used in many products in pharmaceutical as an excipient and formulation aid. It can provide emollient and other performance benefits to the formulation.

For each test, 10 volumes of distilled water grade 3 (ISO 3696) and glycerol have been dispensed with an air displacement pipette noted regular pipette, with a nominal volume of 100 µL and associated tips of 100 µL and a positive displacement pipette, Gilson MICROMAN® E with a nominal volume of 100 µL-M100E, and its appropriated Capillary Pistons, CP100.

Two pipetting modes are used: forward mode and reverse mode. Reverse mode is only used with the air displacement pipette.

The systematic error (inaccuracy) and random error (imprecision) of each pipette were determined according ISO8655 standards.

Principle and pipetting mode description:

The forward mode is the regular aspirate and dispense mode (see figures 1 and 2). In general, the precision of the forward mode relies on precise draining by air pressure (air-displacement pipette) or internal wiping of the pipette barrel (positive-displacement pipette).

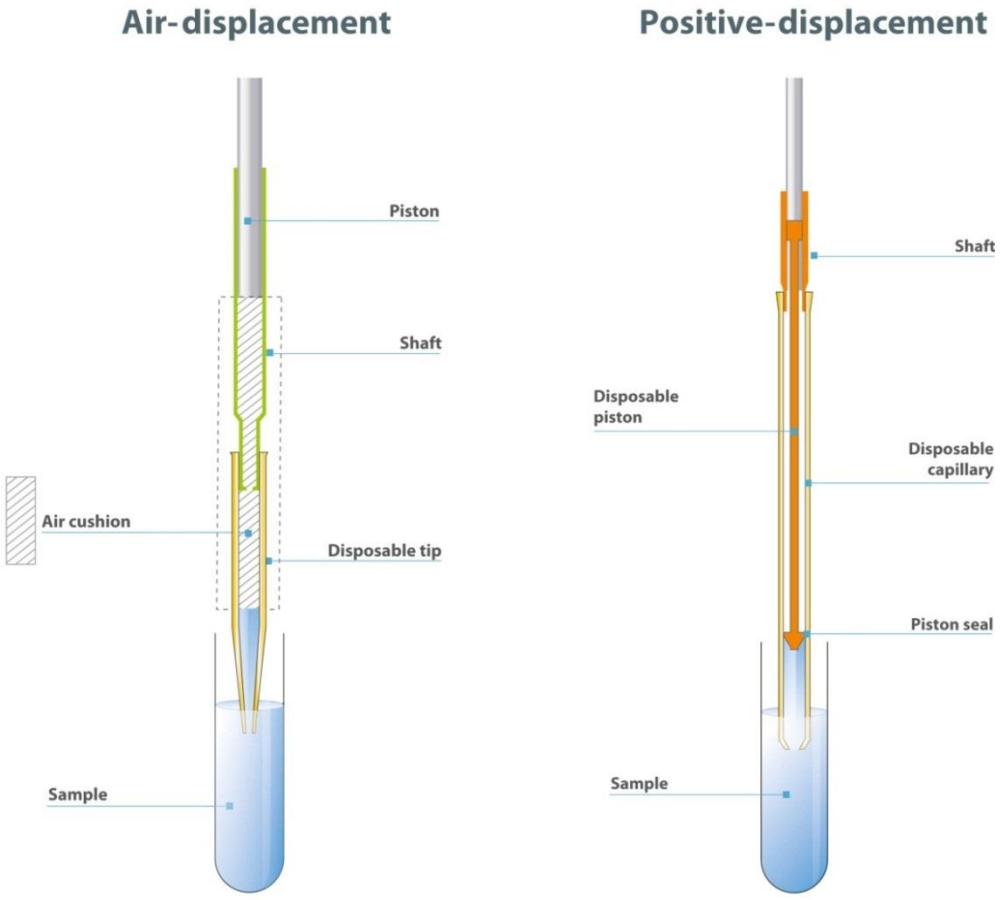

Figure 1: Two pipetting concepts - air and positive-displacement

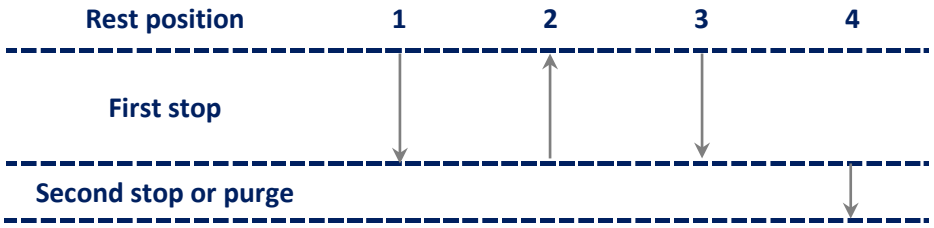

Figure 2: Forward mode pipetting

1. Preparation: Hold the instrument in a nearly vertical position. Depress the plunger smoothly to the first stop position.
2. Aspiration: Immerse the pipette tip in the liquid. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.
3. Distribution: Place the pipette tip at an angle (10 to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position.
4. Purge: Wait one second, and then depress the plunger to the second stop position. This "blow-out" stroke removes any remaining sample from the tip. Remove pipette tip end from sidewall by sliding it up the wall

Forward mode with a positive displacement pipette is similar to the forward mode of air displacement pipette but the purge step is replaced by the ejection of Capillary Pistons.

1. Preparation: Press the plunger button to the first stop. The piston moves to the appropriate position.
2. Aspiration: Immerse the Capillary Piston in the liquid Release the plunger letting it move up to the home position. The piston moves up and the ambient pressure forces the desired volume of liquid through the orifice into the capillary.
3. Distribution: Press the plunger button to the first stop. The piston moves down and expels the liquid out of the capillary.
4. Ejection: Press the plunger all the way down to the second and last stop. Capillary and piston are ejected without hand contact.

Reverse mode pipetting is used for pipetting solutions with a high viscosity or a tendency to foam. The purge stroke is used during preparation. During aspiration, an amount of liquid equal to the amount of purged air is added. This amount compensates for the liquid that remains as film inside the tip during dispensing.

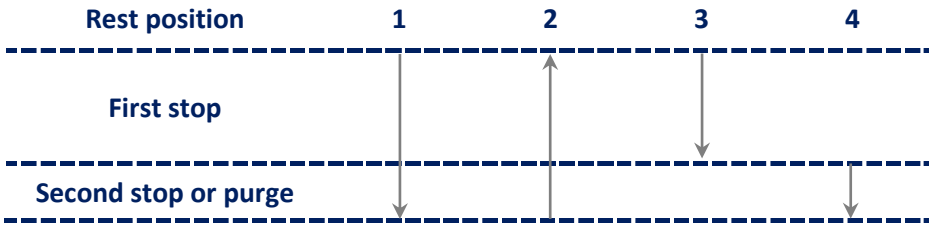

Figure 3: Reverse mode pipetting

1. Preparation: Hold the instrument in a nearly vertical position. Depress the plunger smoothly to the second stop position
2. Aspiration: Immerse the pipette tip in the liquid. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.
3. Distribution: Place the pipette tip at an angle (10 to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position. Wait one second.
4. Re-aspiration: If the pipette tip is to be reused for the same sample, maintain the plunger in the intermediate position for subsequent immersion for the next pipetting cycle and restart operation 2.
5. Complete purge: Wait one second and purge. If the pipette tip is not to be re-used, depress the plunger to purge position over an appropriate waste container and then eject the tip.

Calculation description:

The average volume was determined by gravimetric measurements. The systematic error or inaccuracy of a pipette can be expressed as a percentage of the nominal volume:

$$E\% = (\bar{V} - V0) \times 100 / V0$$

E systematic error
V0 nominal volume
$\bar{V}$ mean volume

A percentage of random error or precision was calculated using this equation:

$$RSD = \frac{SD}{\bar{V}} \times 100$$

with

$$SD = \sqrt{\sum_{i=1}^{n} \frac{(\bar{V} - V_i)^2}{n - 1}}$$

$$\bar{V} = \frac{1}{n} \sum_{i=1}^{n} V_i$$

RSD random error or relative standard deviation
SD Standard deviation
$\mathbf{V_i}$ individually measured volume
n number of measurements
$\bar{\mathbf{V}}$ mean volume

Results and Discussion

During calibration of pipettes, as shown in figure 4, the systematic and random errors for pipetting 100 µL of water are similar for the regular pipette (0.23% and 0.06% respectively) compared to MICROMAN® E (-0.12% and 0.14%).

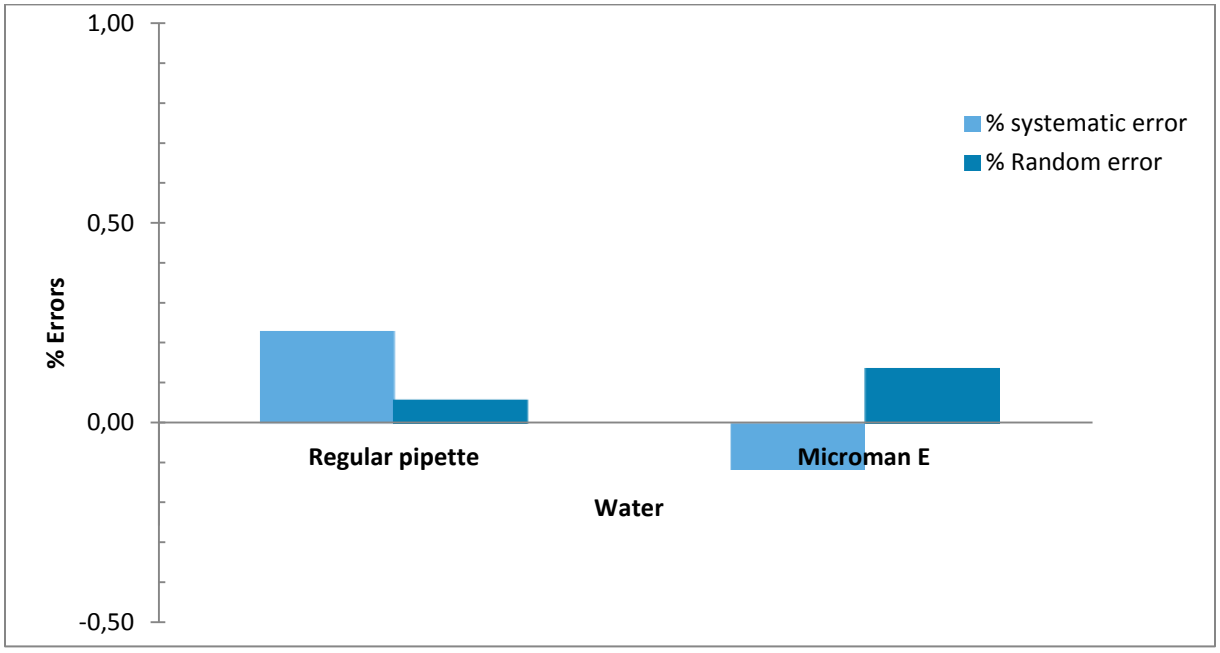

Figure 4: Systematic and random errors of water pipetting with MICROMAN® E vs. regular pipette. Measurements were based on the average of 10 gravimetric measurements per sample.

With Glycerol, as shown in figure 5, the systematic and random errors vary considerably with regular pipette (-4.67% and 3.25% respectively) whereas with MICROMAN E (0.16% and 0.12%) they stay similar to the specifications with water.

Besides, with the regular pipette, the systematic and random errors of the regular pipette in forward mode (-4.67% and 3.25% respectively) are similar to the ones of the reverse mode (-4.38% and 2.54%). These results are higher than ones with MICROMAN E in forward mode (0.16% and 0.12%)

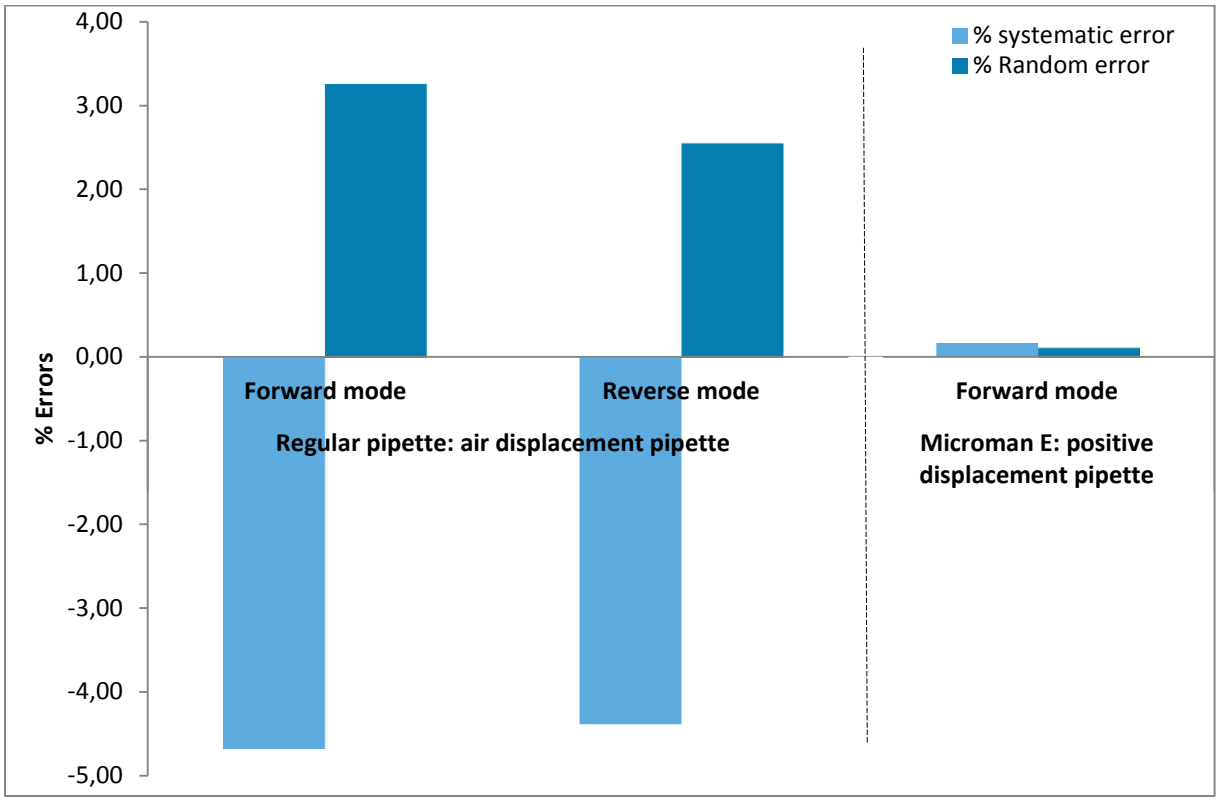

Figure 5: Comparison of systematic and random errors of viscous liquids pipetting with MICROMAN® E in forward mode vs. regular pipette in forward and reverse mode.
Measurements were based on the average of 10 gravimetric measurements per sample.

When accurate and precise dispensing of viscous solutions is critical, a regular pipette is of limited use whatever the pipetting mode. With viscous liquids, the systematic or random errors fall outside the error limits defined for water and the individual measurements vary considerably. In contrast, the results obtained with MICROMAN E are similar with Glycerol and water.

Conclusions

MICROMAN® E, a positive displacement pipette, in combination with Capillary Pistons improves efficiency with viscous liquids pipetting in pharmaceutical formulation. Viscous liquids are fully and quickly dispensed without residual sample left in the tip. When pipetting viscous solutions, the different pipetting modes with air displacement pipette are not recommended, MICROMAN E and Capillary Pistons are the recommended solution to pipette with accuracy and precision.

References

1. Gilson SAS, 2014, N. Belhadj, T. Barthlen, Achieve Pipetting Precision of Problem Liquids with MICROMAN® E
2. Gilson SAS, 2007, Guide to Pipetting