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ViscoTip®: Optimized Performance for Highly Viscous Liquids

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Abstract

Viscous liquids, such as glycerol, are hardly dispensed precise and accurate with classic air-cushion pipetting tools. Therefore, direct displacement tools and according consumables are recommended. However, with increasing viscosity adhesion and cohesion forces increase. Hence, liquid flow into and out of the tool becomes difficult or even impossible even for established tools.
In this application note, we tested a new direct displacement tip specialized for highly viscous liquids (ViscoTip®) and compared the performance with Combitips advanced®. In detail, we measured precision and accuracy when dispensing 99.5 % glycerol using ViscoTip and showed that both, systematic and random error, are within the ISO 8655 error limits. Additionally, we compared 10 mL ViscoTip and 10 mL Combitips advanced in handling various viscous liquids.Combitips advanced could easily handle liquids with a viscosity of up to 200 mPa*s (e.g., ~86 % glycerol). In contrast, ViscoTip could handle also highly viscous liquids (e.g., liquid honey) easily. Furthermore, aspiration and dispensing forces of different viscous liquids usable with Combitips advanced as well as ViscoTip were determined. It was shown that forces were reduced massively by using ViscoTip which protects the user from muscle strain and the dispenser tool from wear-out.

In summary, for liquids with a viscosity of > 200 mPa*s and a volume > 2.5 mL, we recommend to use the ViscoTip for convenient, precise and reliable handling of viscous liquids.

Introduction

Most solutions used in laboratories, such as buffers and growth medium, are aqueous. Additionally, highly viscous liquids such as glycerol or Tween® 20 are commonly used – often as components of buffers. Due to their special flow behavior, these liquids often lead to difficulties in handling. Highly viscous solutions are not only used in the molecular biology laboratory, but also in quality analysis of food (e.g., honey, tomato sauce), cosmetics (e.g., body milk, pigment solution) or cleaning agents (e.g., dishwashing detergent). Furthermore, oils such as mineral oil, palm oil or edible oil need to go through quality control and are transferred with pipettes. Classic air-cushion pipettes reach their limit when viscous solutions need to be transferred precisely and accurately. The liquid can be aspirated hardly and only very slowly. Additionally, residual liquid remains in the tip when dispensing, even with special pipetting techniques such as reverse pipetting. Positive displacement devices, working like a syringe, offer easier handling of viscous solutions up to their individual limit. At a certain degree of viscosity, the precision and accuracy generally decreases and the aspiration and dispensing forces increase. Since no accurate and precise transferring tool for highly viscous solutions above 2.5 mL is available, most laboratories weigh their samples and calculate the sample volume by considering liquid density. Viscosity is an important parameter of a solution and has an impact on aspiration and dispensing properties. It can be determined by different types of viscosimeters, most of them measuring the liquid movement, called dynamic viscosity. Dynamic viscosity is determined as the force needed by a liquid to overcome its own internal molecular friction to flow. It's expressed in the measurement unit mPa*s (milli Pascal per second) [1, 2]. Viscous liquids are classified into newtonian and non-newtonian liquids, which behave differently when pressurized or stirred. A newtonian liquid has a constant viscosity that is independent from outer influences such as pressure. One example is water. A non-newtonian liquid can either get harder, or more fluid when moved or pressurized. Ketchup (Tomato sauce) gets more fluid when stirred or moved, that is why shaking a ketchup bottle leads to fast flow out of the sauce. Oobleck (corn starch dissolved in water) is usually a thick liquid but gets solid under pressure [3]. Even grown-ups can walk on the pressurized starch-solution and still, easing the pressure, it will flow like water through the fingers.

In this application note we tested and compared ViscoTip and Combitips advanced with various viscous liquids, focusing on newtonian liquids because of homogenous properties. We measured precision, accuracy and forces needed for aspiration and dispensing. Aim of the study was to define a viscosity threshold at which a switch from a standard dispenser tip, such as Combitips advanced, to a ViscoTip is recommended to assure precise and accurate handling of the liquid.

Material and Methods

Determination of error limits with 99.5 % glycerol
The systematic and random error of ViscoTip were determined gravimetrically using 99.5 % anhydrous glycerol. Measurements were performed according to ISO 8655 standards.

Because error limits for glycerol do not exist, the limits for distilled water stated in ISO 8655 [4] and the stricter Eppendorf error limits of 10 mL Combitips advanced [5] were used as reference (table 1).

Table 1: Error limits for systematic and random error using distilled water per ISO 8655 and Eppendorf error limits

Specifications		Systematic error limits for distilled water [%]		Random error limits for distilled water [%]	
		Eppendorf	ISO 8655	Eppendorf	ISO 8655
Multipette®/Repeater® M4	200 µL	± 0.5	± 8.0	± 0.6	± 4.0
	1,000 µL	± 0.5	± 1.6	± 0.4	± 0.8
	2,000 µL	± 0.5	± 0.8	± 0.3	± 0.4
Multipette®/Repeater® E3x	1,000 µL	± 0.5	± 5.0	± 0.25	± 3.0
	5,000 µL	± 0.4	± 1.0	± 0.25	± 0.6
	10,000 µL	± 0.4	± 0.5	± 0.15	± 0.3

Three Multipette/Repeater M4 and three Multipette/Repeater E3x were tested.
For Multipette/Repeater E3x speed level 1 was used for aspiration and speed level 2 for dispensing.
The balance used was model XS205 DU (Mettler Toledo®, USA).

Determination of correct dispensing volumes using various viscous liquids
The correct dispensing volume of 10 mL Combitips advanced and 10 mL ViscoTip was determined gravimetrically using various viscous liquids (table 2). For commercially available liquids, viscosity was determined using the viscosimeter PCE-RVI1 (PCE Instruments, Germany). Multipette/Repeater E3x was used for testing. Speed level 5 was selected for aspiration and dispensing. For liquids showing very slow flow behavior (e.g., liquid honey) speed levels were reduced to 2 for proper liquid handling.

Combitips advanced and ViscoTip were pre-wetted once prior to each measurement.
The balance used was model WXTS205 DU (Mettler Toledo, USA).

Table 2: Liquids used for testing correct dispensing volumes and/or aspiration and dispensing forces

Liquid	Supplier	Dynamic viscosity (mPa*s)
Mineral oil	Sigma® (Art.-Nr. M5904-500ML, Lot 068K0057)	26 (23 °C)
Motor oil Helix Ultra Professional 5W-30	Shell®	130 (23 °C)
Paraffinum oil	Roth (Art.-Nr. 8904.1, Charge 401177175)	200 (20 °C)
Triton® X-100	Roth (Art.-Nr. 3051.3, Charge 305227705)	240 (25 °C)
Tween® 20	Merck® (8.22184.0500, Charge S4571884 628)	400 (25 °C)
99.5 % glycerol (ROTIPURAN® p.a.)	Roth (Art. 3783.1, Charge 356249010)	1,412 (20 °C)
Viscosity standard APN1400	Paragon Scientific Ltd (R) (Lot 5123110, Certificate No.: AP1717)	5,041 (20 °C) 3,418 (25 °C)
Liquid honey (Langnese Flotte Biene® Wildblütenhonig)	Langnese Honig (Charge LH60776)	12,500 (22 °C)
Hand Cream (Dualin)	Peter Greven Physioderm	13,600 (23 °C)
Viscosity standard APN4000	Paragon Scientific Ltd (Lot 4131806, Certificate No.: APP1840)	14,042 (20 °C) 9,256 (25 °C)

Comparison of aspiration and dispensing forces of various viscous liquids

Aspiration and dispensing forces for liquids usable with Combitips advanced and ViscoTip from the previous measurement was done using the digital force gauge FH 100 (Sauter GmbH).

The force gauge was attached to the ViscoTip piston and pulled/pushed manually. 5 mL of each liquid were aspirated and dispensed 5 times. Then the arithmetic mean was calculated.

Results and Discussion

Systematic and random error using glycerol 99.5% are within ISO limits for distilled water

Using classic dispensing tips glycerol 99.5% is among the most frequent and yet highly challenging liquids in the lab. In our test series, the systematic and random error using Multipette/Repeater M4 with ViscoTip for dispensing 99.5 % glycerol were well within the ISO 8655 error limits defined for distilled water (table 3). Furthermore, error limits were also within the much stricter Eppendorf error limits for positive displacement dispenser. Using a Multipette/Repeater E3x with ViscoTip the error limits comply to ISO 8655 for distilled water. In conclusion, the results show that ViscoTip is as precise and accurate when dispensing 99.5% glycerol as when using other direct displacement tips with distilled water.

Table 3: Systematic and random error limits of ViscoTip using 99.5 % glycerol compared to reference values for distilled water

Specifications		Systematic error (99.5 % glycerol; n=10)	Systematic error limits for distilled water [%]	
			Eppendorf	ISO 8655
Multipette/Repeater M4	200 µL	-0.3	± 0.5	± 8.0
	1,000 µL	-0.3	± 0.5	± 1.6
	2,000 µL	-0.3	± 0.5	± 0.8
Multipette/Repeater E3x	1,000 µL	-0.3	± 0.5	± 5.0
	5,000 µL	-0.3	± 0.4	± 1.0
	10,000 µL	-0.3	± 0.4	± 0.5
		Random error limits (99.5 % glycerol; n=10)	**Random error limits for distilled water [%]**	
			Eppendorf	ISO 8655
Multipette/Repeater M4	200 µL	0.5	± 0.6	± 4.0
	1,000 µL	0.3	± 0.4	± 0.8
	2,000 µL	0.2	± 0.3	± 0.4
Multipette/Repeater E3x	1,000 µL	0.3	± 0.25	± 3.0
	5,000 µL	0.2	± 0.25	± 0.6
	10,000 µL	0.1	± 0.15	± 0.3

The ViscoTip outperforms classic tips using various viscous liquids

Using 10 mL Combitips advanced with various viscous liquids, it was shown that a limit in handling is reached at a dynamic viscosity around 200 mPa*s. Liquids with a higher viscosity cannot be dispensed precisely anymore. Some of the tested liquids could not be aspirated or dispensed at all (table 4).

In contrast ViscoTip used for dispensing various viscous liquids enable dispensing the correct liquid volume. Each tip was pre-wetted prior the measurement and our tests showed that pre-wetting is essential to reach reliable and repeatable results using ViscoTip.

Table 4: Comparison of usability for various viscous liquids using Combitips advanced and ViscoTip.

Liquid	Dynamic viscosity in mPa*s	Combitip Advanced 10 mL	ViscoTip 10 mL
Mineral oil	26 (23 °C)	✓	✓
Motor oil	130 (23 °C)	✓	✓
Paraffinum oil	200 (20°C)	✓	✓
Triton X-100	240 (25°C)	(✓)	✓
Tween 20	400 (25°C)	(✓)	✓
Viscosity standard APN1400	5,041 (20°C) 3,418 (25°C)	X	✓
Liquid honey	12,500 (22°C)	X	✓
Hand cream	13,600 (23 °C)	X	✓
Viscosity standard APN4000	14,042 (20°C) 9,256 (25°C)	X	✓

✓ Dispensed liquid volumes were correct
X Dispensed liquid volumes were incorrect or aspiration/dispensing impossible
(✓) Error message in the last dispensing step

We could show that a broad range of liquids can be handled using ViscoTip ranging from <200 up to 14,000 mPa*s. In comparison, Combitips advanced 10 mL generally showed a limit of handling viscosity at approx. 200 mPa*s. For Tween 20 and Triton X-100 the last dispensing step using Combitips advanced was incorrect or led to an error message. The pressure building up in the system was too high and the Multipette/Repeater E3x stopped to avoid motor damage. With Tween 20 and Triton X-100 we recommend using ViscoTip or to discard the last dispensing step when using Combitips advanced. In contrast, all tested liquids could be aspirated and dispensed using ViscoTip. The construction of ViscoTip allows for easy aspiration and dispensing and correct dispensing results.

The ViscoTip reduces aspiration and dispensing forces

Another important parameter when pipetting viscous liquids is the operating force needed to aspirate and dispense the liquid. High forces can stress the arm muscles when using manual dispensers leading to repetitive strain injury. When using electronic dispensers, the motor may overheat or strike at high operating forces. Furthermore, high operating forces can lead to a reduced battery operating time. This is prevented in Eppendorf dispensers by the motor being switched off automatically at a critical value.

The liquids that showed correct dispensing volumes in the previous test were used to determine operating forces with Combitips advanced as well. Using ViscoTip it was shown that aspiration and dispensing forces were highly reduced compared to Combitips advanced. Aspiration force was reduced up to 74 % while dispensing force was reduced up to 71 % when using mineral oil. The most viscous liquid usable with both tips was Tween 20. Aspiration force was reduced 66 % while dispensing force was reduced 51 % when using ViscoTip instead of Combitips advanced (figure 1). As a result, we recommend using ViscoTip for liquids that could be used with both tip variants to achieve the best results with least effort.

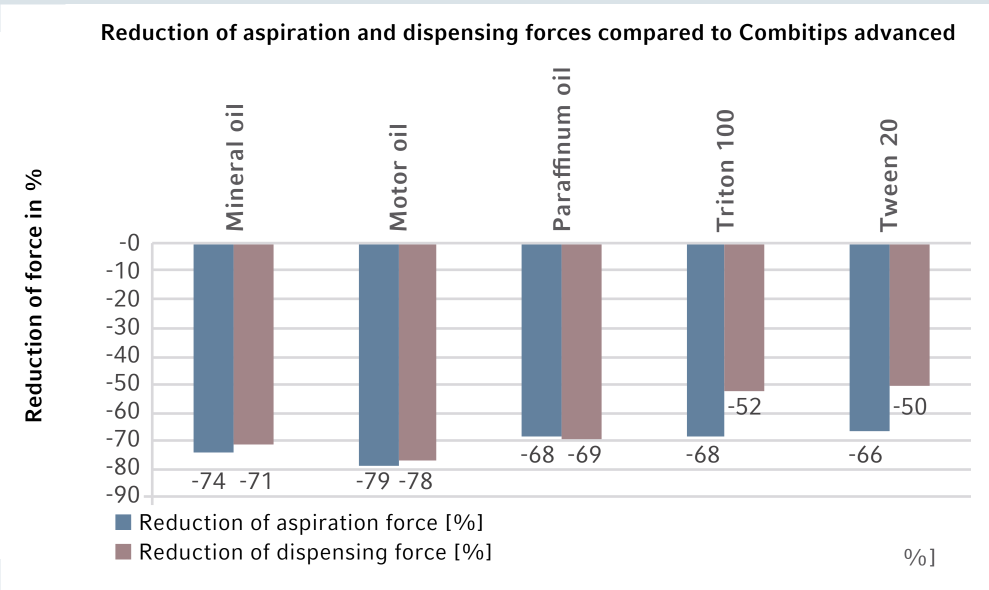

Figure 1: Reduction of forces when aspirating/dispensing liquids using ViscoTip compared to Combitips advanced (baseline). n=3

Previous tests (data not shown) showed that, in contrast to volume variants used in this study, Combitips advanced versions ≤ 2.5 mL can be used to dispense liquids with a viscosity of up to 840 mPa*s precisely and accurately. Due to the smaller volumes, less force is required for aspiration and dispensing of viscous liquids.

Overall the results of this previous study and this application note show that ViscoTip simplify handling of viscous liquids > 2.5 mL volume by reducing aspiration and dispensing forces. Reduced forces lead to more ergonomic working and reduce stress on wear-out parts of manual and electronic dispensers. We recommend using Combitips advanced 0.1 mL – 2.5 mL for viscous liquids up to 840 mPa*s and larger Combitip advanced volumes only up to 200 mPa*s. For larger volumes of highly viscous liquids we recommend ViscoTip (figure 2).

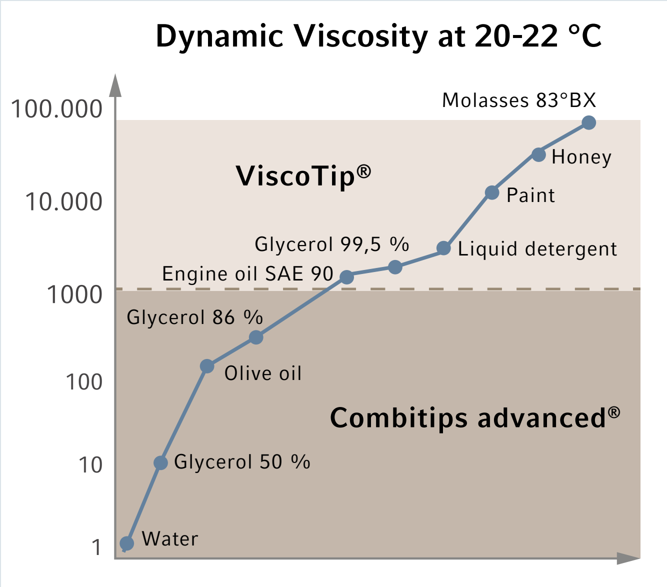

Figure 2: Combitips advanced can be used for liquids with a viscosity up to 840 mPa*s. For liquids with a higher viscosity we recommend using ViscoTip.

Conclusion

The results of testing various viscous standards and laboratory solutions, as well as viscous food, cosmetics and oils showed that using ViscoTip is beneficial in multiple aspects. ViscoTip could handle viscous liquids up to 14,000 mPa*s. Furthermore, it complied to error limits stated by ISO 8655 and the stricter Eppendorf error limits when dispensing 99.5 % glycerol. In contrast, 10 mL Combitips advanced had a limit of handling viscous solutions at around 200 mPa*s. Additionally, aspiration and dispensing forces for viscous liquids usable with Combitips advanced and ViscoTip were determined. It was shown that less force is needed when using ViscoTip. This protects manual and electronic dispenser from wearing out and the user from muscular stress when using manual dispenser. In general, we recommend using 10 mL Combitips advanced for liquids with a maximum viscosity of 200 mPa*s (~ 86 % glycerol at 20 °C). For liquids of higher viscosity, Combitips advanced smaller than 2.5 mL or ViscoTip must be used to maintain correct dispensing results.
In conclusion, ViscoTip are the optimal choice to handle volumes of more than 2.5 mL of viscous liquids.

Literature

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[3] Soundararajan H N, Agustsson E, Diaz Martinez R A, Westergren A, Gonzalez-Conde Perez J L. Usage of Oobleck as a packaging material. International Journal of Scientific and Research Publications. 2013. Volume 3, Issue 3.
[4] EN ISO 8655, Parts 1-6: Piston-operated volumetric apparatus. © ISO 2002.
[5] Standard Operating Procedure. www.eppendorf.com

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Ordering information

Product description	Order no. international	Order no. North America
Multipette®/Repeater® E3, single-channel, with charging cable and Combitips advanced® assortment pack (1 Combitip of each size), 1 µL – 50 mL	4987 000.010	4987000010
Multipette®/Repeater® E3x, single-channel, with charging cable and Combitips advanced® assortment pack (1 Combitip of each size), 1 µL – 50 mL	4987 000.029	4987000029
ViscoTip®, 10 mL	0030 089.502	0030089502

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