Applications

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Technical Report

Dispensing of highly viscous liquids

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Abstract

Based on their physical properties, which differ from those of water, viscous liquids (e.g. glycerol) present a particular challenge to dispensing systems. Here, the size of the air cushion located between the piston and the surface of the liquid is of particular importance. With a positive displacement system such as the Multipette® (Repeater), even highly viscous fluids can be dispensed as accurately as water. The electronic positive displacement systems available on the market differ in their ability to dispense highly viscous liquids; the factors "size of dispenser tip", "viscosity of liquid", as well as "selected aspiration speed and dispensing speed" are of particular significance here.

Introduction

Non-standard liquids in the laboratory

Modern laboratory applications – in research as well as analytical laboratories – require increased handling of liquids whose physical properties differ from those of water. The following factors play a major role [1]:

– density (e.g. sulfuric acid),
– vapor pressure (e.g. acetone),
– surface tension (e.g. detergent containing aqueous solutions),
– viscosity (e.g. glycerol).

In accordance with ISO 8655 [2], air cushion pipettes are calibrated to the dispensing of water. Dispensing of liquids with different physical properties is (despite high precision of the pipette when using water) usually faulty. The dispensing error rises with increasing differences of the liquid to the physical properties of water and is characterized by systematic as well as random errors. To this end, in order to maintain precision, pipettes need to be adjusted (e.g. for altered density), or handling technique needs to be modified to meet the requirements of the liquids to be dispensed [3]. With regards to pipetting of, for example, viscous liquids, mainly slow pipetting speed, as well as reverse pipetting, are applied.

During reverse pipetting, the stroke button is depressed completely, and the liquid is aspirated including the blow-out volume. Subsequently, dispensing occurs using only the regular stroke, i.e. the button is depressed only to the first "Stop", and residual liquid will remain inside the tip [4]. However, these measures are only sufficient when liquids with minimal deviations from the physical properties of water are to be dispensed (e.g. dilutions). In most cases, modified pipetting techniques or pipette adjustment will not guarantee precision which is comparable to the pipetting of water. In these cases it is advisable to abandon the air cushion principle and instead choose a dispensing system of the positive displacement principle (dispensers or positive displacement pipettes).

Viscous liquids

Viscosity is a property based on the internal friction within a liquid. The viscosity of a liquid is characterized as dynamic or kinematic viscosity. Kinematic viscosity is expressed in [m^2/s] or Centistokes [cSt], respectively. Dynamic viscosity, in accordance with SI unit (International System of Units), is expressed in [mPa*s], or, according to CGS (Centimeter-Gram-Second-System), in Poise [P] or Centipoise [cP], respectively. Thereby 1 mPa*s = 1 cP. Under consideration of density, kinematic viscosity and dynamic viscosity may be converted into each other [5].

The viscosity of a liquid is highly dependent on its temperature. Viscosity increases with decreasing temperature. The viscosity values of 100 % glycerol at different temperatures (shown in table 1) illustrate the extent of this effect. Generally, most lab personnel are not familiar with details regarding the viscosity of a liquid, and the viscosity of the liquids to be dispensed (especially during dilutions) is unknown. In order to allow for a rough estimate of magnitude, the dynamic viscosity values for different materials are listed in table 1.

Table 1: Dynamic viscosity values of different media

Medium	Temperature [°C]	Dynamic Viscosity [mPa*s]
Milk	20	2
Canola oil	20	60
Olive oil	20	107.5
Liquid soap (here: eco-clean)	20	600–1,000
Paint stripper	20	5,000
Emulsion paint (here: Maxit, Dinova®)	20	13,000–20,000
Wood varnish (here: Mega Holzlasur ML)	23	200
Turpentine	20	1.46
Petroleum	20	0.65
Engine oil	20	100–600
Chloroform	20	0.56
Glycerol 100 %	20	1,410
Glycerol 100 %	10	3,900
Glycerol 100 %	0	12,070
PEG-200 (MW 190–210)	25	50
PEG-300 (MW 280–320)	25	70
PEG-400 (MW 380–420)	25	90
PEG-600 (MW 570–630)	25	135
Tween® 20	25	250–450
Triton® X-100	25	240

Note: These values are guidelines only. Viscosity may vary according to preparation of the medium.

One liquid of high viscosity, which is frequently used in laboratories, is glycerol (IUPAC: Propane-1,2,3-triol). This substance is characterized by a concentration-dependent increase in viscosity (Fig. 1) [6]. Its physical properties are listed in table 2.

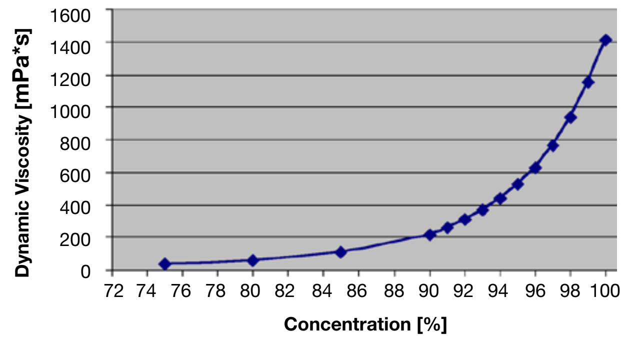

Fig. 1: Connection between viscosity and concentration at 75 % to 100 % glycerol, 20 °C [6]

Table 2: Physical properties of water and 100 % Glycerol at 20 °C

Physical property	Water (double distilled)	100 % Glycerol
Density	1 g/cm³	1.26 g/cm³
Vapor pressure	23 hPa	< 0.001 hPa
Viscosity	1 mPa*s	1410 mPa*s

When using an air cushion pipette for dispensing highly concentrated glycerol, considerable dispensing errors may occur despite pipette adjustment and modified pipetting techniques (very slow working speed, reverse pipetting). This is due to a lesser degree to the elevated density of glycerol, but rather decreased flow dynamics. During aspiration, air bubbles are often formed within the aspirated volume, and during dispensing a liquid film remains inside the tip.
In contrast, when using a direct replacement system, in addition to a drastically reduced air cushion volume (thus achieving noticeable reduction in errors due to the air cushion), the piston, which is integrated into the tip of a direct displacer, allows for accurate dispensing of liquid by preventing fluid retention on the inside wall of the tip. Thus, even high percentage glycerol solutions may be dispensed with high precision, compared to using air cushion pipettes.
A further advantage provided by electronic dispensing instruments, which provide motor-controlled piston movement, is that the working speed remains constant at all times. This is of special significance during the dispensing of highly viscous solutions, as under these circumstances, precision is very sensitive to too fast or variable pipetting speed.
Furthermore, ergonomic considerations favor the use of electronic dispensing devices, since the decreased flow dynamics of viscous liquids require increased force during aspiration and dispensing. Using an electronic dispenser with motor control, this force is not re-directed to the user.
This publication describes the beneficial effects of the reduced air cushion of positive displacement instruments compared to the traditional air cushion pipette with and without modified pipetting technique (reverse pipetting, very slow working speed) during pipetting of highly viscous liquids. Furthermore, the ability to dispense different glycerol concentrations using electronic hand dispensers available on the market is examined. The aim of this investigation is to determine the maximum viscosity which may be dispensed using these instruments.

Materials and Methods

All pipetting steps were performed in accordance with ISO 8655 [2] or the Eppendorf SOP [7], respectively.
All instruments were used in combination with tips made by their respective manufacturer. In order to prepare the different viscosities, 99.6 % glycerol (Fluka, Lot #: 1344661, filling code: 53507327) was diluted with distilled water, and the resulting viscosities were determined using a Stabinger viscosimeter:
75 % (43.95 mPa*s), 85 % (121.7 mPa*s),
90 % (227.0 mPa*s), 92 % (291.5 mPa*s),
94 % (392.6 mPa*s), 96 % (530.6 mPa*s),
98 % (712.1 mPa*s), 99.6 % (835.8 mPa*s).

Dispensing of 90 % glycerol with pipette and hand dispensers

Within the investigations presented here, the pipettes Eppendorf Reference® 100 µl fix (tip size: 20–200 µl) and Eppendorf Research® 100–1000 µl (tip size 50–1000 µl) were used, as well as the positive displacement instruments Eppendorf Multipette® plus (Repeater plus) (manual) and Eppendorf Multipette Xstream (Repeater Xstream) (electronic), equipped with 1 ml Eppendorf Combitip® plus, respectively. Both air cushion pipettes were adjusted to 100 µl for the purpose of pipetting 90 % glycerol. During use of all manual instruments, careful attention was paid to very slow working speed in order to guarantee complete aspiration of the glycerol solution into the tip, as well as correct dispensing. The Multipette Xstream was set to medium piston speed (setting 5). The measurements were performed using an analytical balance (Sartorius AG, Genius ME215-P).
In order to reduce evaporation of the test fluid during measurements, a humidity trap was employed (Sartorius AG, within the pipette calibration set YCP03-1). The temperature inside the measuring room was 20 °C; prior to each measurement, pipette tips were pre-wetted 5 times, and dispenser tips were pre-wetted once. For determination of systematic and random errors, 10 pipetting steps of 100 µl each were performed. Under consideration of the Z-factor, the errors were subsequently calculated from the individual measurements. To this end, the calibration software PICASO, version 2.2 (Eppendorf) was used.

Electronic hand dispensers: maximum dispensable viscosity

Four different electronic dispensers were used in the examination of maximum dispensable viscosity: Multipette Xstream (software: main: 02.03.00, motor: 01.11.21), as well as instruments made by other manufacturers (A, B, C). In order to achieve the highest possible capacity, the instruments were used as pipettes, and the respective maximum possible volume was aspirated/dispensed (no dispensing of partial volumes). Dispensing was performed at lowest, medium and highest piston speed. However, differences in speed between instruments made by different manufacturers need to be taken into consideration, particularly in the slowest range.
In contrast to the Multipette Xstream, which features 10 speed settings, the dispensers made by manufacturers A, B and C are limited to three speed settings. For this reason, more detailed considerations of piston speed were not possible. With the exception of manufacturer C (no production of sterile tips), all measurements were performed with sterilized dispenser tips, as irradiated products tend to display elevated friction of the piston against the cylinder. Each dispenser tip was pre-wetted once. Subsequently, three dispensing steps were performed. Only when all three dispensing steps were completed without error messages, the respective combination of the factors dispensing volume/tip size/viscosity/piston speed was considered valid. The temperature inside the measuring room was 23.0 °C – 24.0 °C.

Results and Discussion

1.1 Dispensing of 90 % glycerol and distilled water using air cushion pipettes

Within these experiments, 100 µl of a 90 % glycerol solution were aspirated using either a 100 µl pipette (Eppendorf Reference) or a 100–1000 µl pipette (Eppendorf Research). In order to determine the difference between the two pipetting techniques, pipetting steps were performed by "forward pipetting" as well as "reverse pipetting". For comparison, a 100 µl Reference pipette was employed for dispensing distilled water using normal pipetting technique.

Figure 2 depicts the dispensing results using the 100 µl Reference pipette, and figure 3 shows dispensing results using the 100–1000 µl Research pipette. Both graphs clearly show that pipetting of highly concentrated glycerol using air cushion pipettes will lead to considerable variations in the volumes measured. The amplitudes of variation are independent of the type of pipette (fixed volume vs. adjustable pipette), but are mainly caused by the viscosity of the fluid, as well as the size of the air cushion between the pipette and the surface of the liquid.

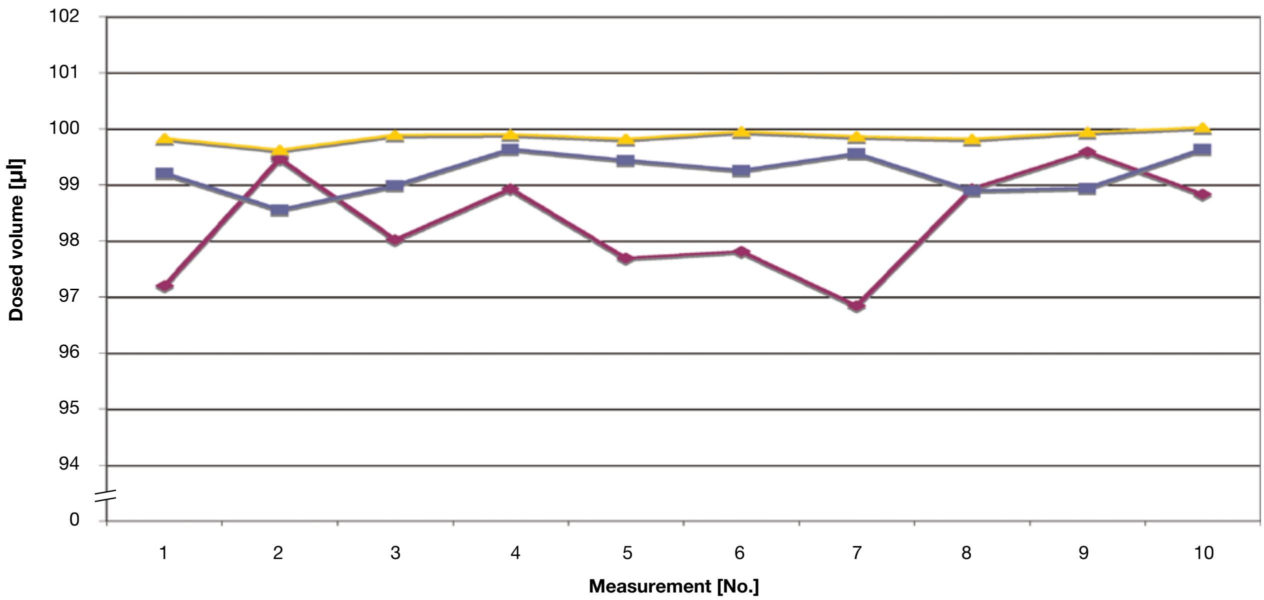

Fig. 2: Dispensing of 90 % glycerol and distilled water using an air cushion pipette (Reference fix). Dispensed volume: 100 µl. Tip size used: 20–200 µl. Legend: FP = forward pipetting; RP = reverse pipetting.

FP Reference 100 µl fix
RP Reference 100 µl fix
FP Reference 100 µl fix A. dest.

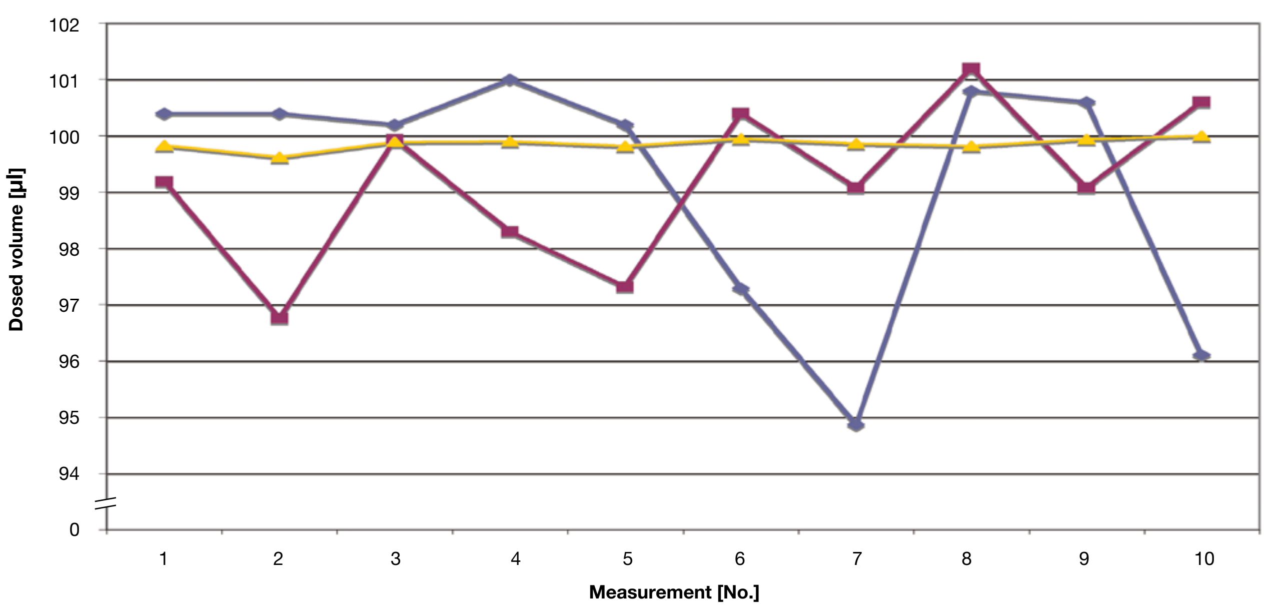

Fig. 3: Dispensing of 90 % glycerol (Research adjustable-volume 100–1000 µl, tip used: 50–1000 µl) and distilled water (Reference fix 100 µl, tip used: 20–200 µl). Dispensed volume: 100 µl. Legend: FP = forward pipetting; RP = reverse pipetting.

FP Reference 100–1000 µl
RP Reference 100–1000 µl
FP Reference 100 µl fix A. dest.

Thus, comparison of the dispensing error during the use of 20–200 µl tips and 50–1000 µl tips (Fig. 2 and 3) illustrates the influence of air cushion size on pipetting accuracy: During forward as well as reverse pipetting the 1000 µl tip shows greater variation than the smaller 200 µl tip. This result is verified by the systematic and random errors calculated from the 10 individually measured values (not shown). Figures 2 and 3 further illustrate the influence of pipetting technique on accuracy. The technique of reverse pipetting is able to reduce variability of the dispensing results compared to forward pipetting; however, the errors fall outside the error tolerance for the systematic and random errors of the pipette used (not shown).

1.2 Dispensing of 90 % glycerol using hand dispensers
Positive displacement instruments such as the Multipette plus (manual) or Multipette Xstream (electronic) do not show the variations in measured values observed with pipettes (Fig. 4). Instead, the dispensing accuracy is comparable to pipetting distilled water using an air cushion pipette (here: Reference fix 100 µl).

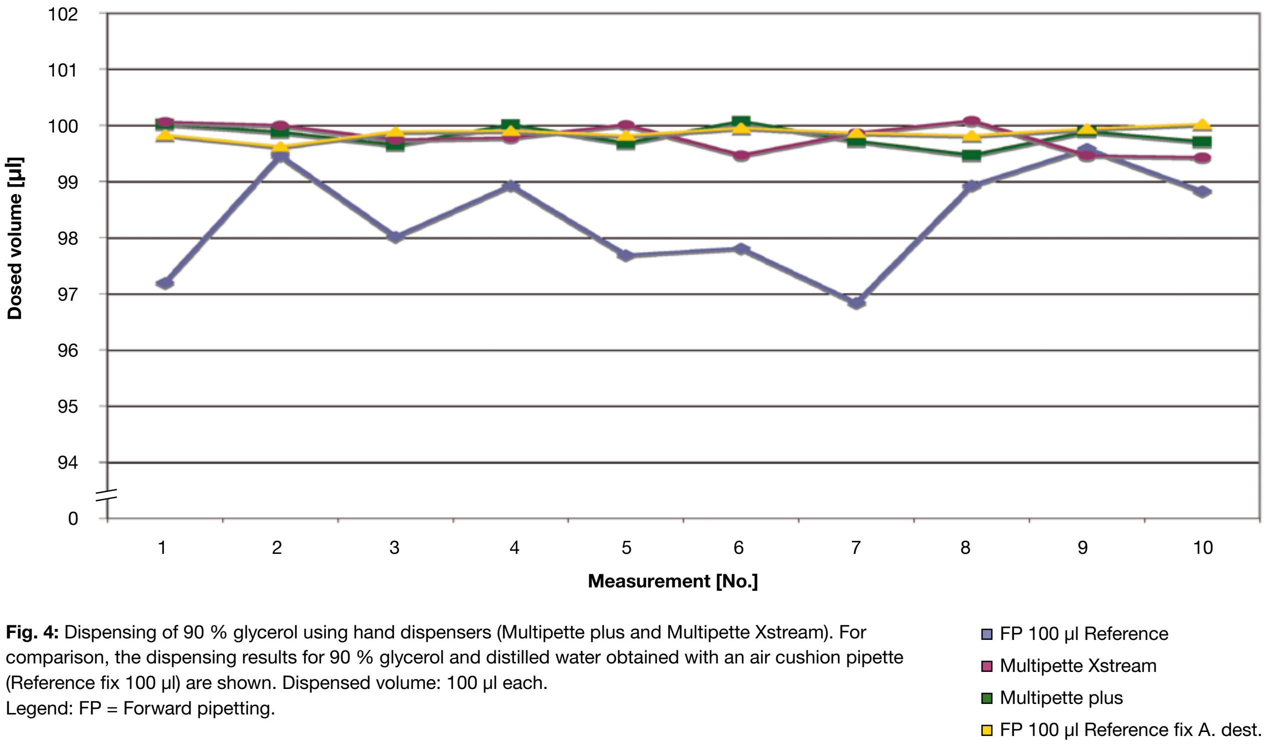

Fig. 4: Dispensing of 90 % glycerol using hand dispensers (Multipette plus and Multipette Xstream). For comparison, the dispensing results for 90 % glycerol and distilled water obtained with an air cushion pipette (Reference fix 100 µl) are shown. Dispensed volume: 100 µl each.
Legend: FP = Forward pipetting.

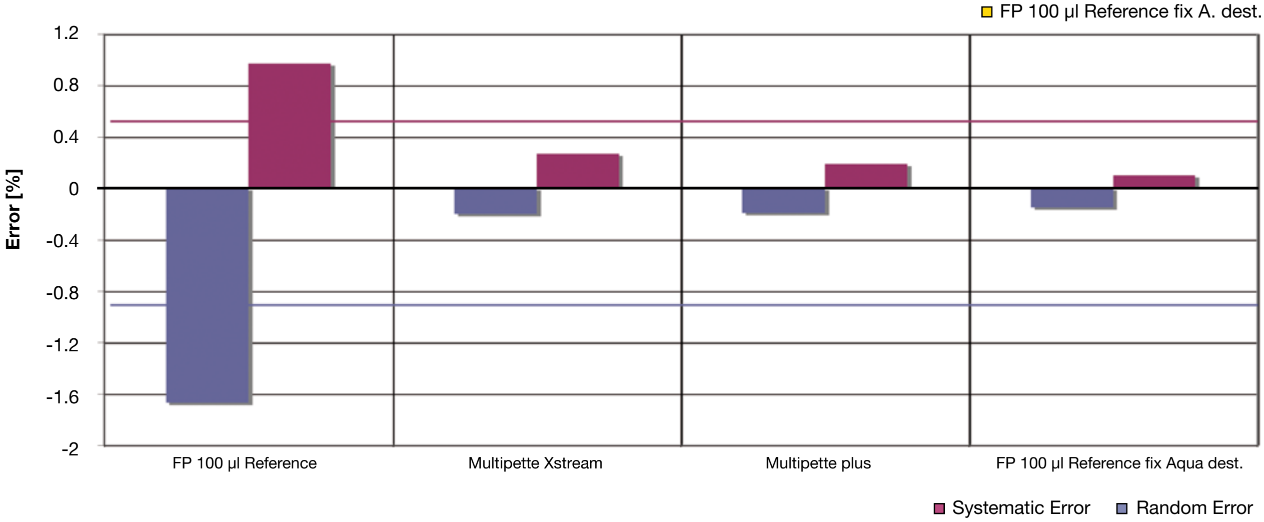

Fig. 5: Systematic and random errors when dispensing 90 % glycerol using hand dispensers (Multipette plus and Multipette Xstream) or an air cushion pipette (Reference fix 100 µl). For comparison, the pipetting errors with distilled water, using the Reference 100 µl fix pipette, are shown.
Dispensing volumes: 100 µl each. Legend: FP = Forward pipetting.
The lines illustrate error limits for the systematic error (lower limit, blue, ± 0.9 %) as well as the random error (upper limit, red, <0.55 %) of the Multipette Xstream with 1 ml Combitip plus.

In contrast to the pipettes, systematic and random errors of both Multipettes are within the error limits of the Multipette Xstream when dispensing 90 % glycerol (Fig. 5). Hence, positive displacement instruments allow for dispensing of liquids with elevated viscosity with equal accuracy as water with a pipette.

2. Electronic dispensers: Maximum dispensable viscosity

The purpose of this investigation was to demonstrate how well commercially available electronic dispensers live up to the challenge of "high viscosity". Due to the low flow dynamics of viscous liquids and thus higher effort needed during dispensing, the dispensers are quite heavily burdened. In cases of excess burden, the instruments will show an error message, which leads to interruption of the current dispensing series. Table 3 lists the results of dispensing different viscosities (glycerol concentrations) with different dispenser tips. In each experiment, the maximum volume of the tip was dispensed. The second table reflects the viscosities and respective glycerol concentrations at 24 °C.

Table 3: Determination of highest dynamic viscosity [mPa*s] which may be dispensed using the different tip sizes; viscosity values indicated are at 24 °C.

Tip size [ml]	Multipette Xstream	Manufacturer A	Manufacturer B	Manufacturer C
0.1	836	836	836	836
0.2	836	–	836	836
0.5	836	836	836	836
1	836	–	836	836
1.25	–	836	–	–
2.5	836	836	836	836
5	836	836	531	712
10	292	–	44	44
12.5	–	393	–	–
25	292	122	44 / n.p.	44
50	122	122	44 / n.p.	44 / n.p.

Concentration [%]	Viscosity at 24 °C [mPa*s]
99.6	836
98	712
96	531
94	393
92	292
85	122
75	44

Legend:
n.p. = Dispensing was not possible at the indicated concentrations (error message).
– = Tip sizes not available from respective manufacturer.

Within this investigation, the highest dynamic viscosity of 836 mPa*s could be dispensed up to a tip size of 5 ml (Multipette Xstream and manufacturer A) and 2.5 ml (manufacturer B and manufacturer C), respectively (Table 3). If larger tips or volumes were used, only glycerol concentrations of lower viscosity could be dispensed. The maximum dispensable viscosity dropped considerably for manufacturers B and C when dispensing tips larger than 5 ml were tested.

Even though manufacturer C offers only non-sterile tips (less friction than sterile tips), only the lowest tested viscosity of 44 mPa*s could be dispensed without an error message. In contrast, the Multipette Xstream, equipped with this tip size and sterile tips, was able to dispense a viscosity of 292 mPa*s. This is equivalent to a difference in viscosity of 248 mPa*s. With regards to the 25 ml tips, the Multipette Xstream enabled dispensing of liquids up to a viscosity of 292 mPa*s, whereas the other instruments reached a maximum of 122 and 44 mPa*s, respectively, at this tip size. Dispensing of 50 ml glycerol solution of the lowest viscosity (44 mPa*s) was not possible without error messages when using instruments made by manufacturers B and C. Using the Multipette Xstream as well as the instrument by manufacturer A with the largest tip (50 ml), the maximum dispensable viscosity was 122 mPa*s.

All manufacturers offer a choice of piston speed. Independent of the instrument-specific differences in piston speed, the ability to dispense was tested at highest, medium and lowest speed. Table 4 shows the highest respective piston speed. Here, the values refer to the maximum dispensable viscosities shown in table 3.

Table 4: Maximum piston speed which may be selected for dispensing of the highest possible viscosity [mPa*s].

Tip size [ml]	Multipette Xstream	Manufacturer A	Manufacturer B	Manufacturer C
0.1	+++	+++	+++	+++
0.2	+++	–	+++	+++
0.5	+++	+++	+++	+++
1	+++	–	+++	+++
1.25	–	+++	–	–
2.5	+++	+++	+++	+++
5	++	+	+++	+++
10	+	–	+++	+++
12.5	–	+	–	–
25	+	++	n.p.	+++
50	+	+	n.p.	n.p.

Legend:
n.p. = Dispensing not possible at concentrations indicated in table 3 (error message);
– = tip sizes not available from this manufacturer.
+++ = highest speed
++ = medium speed
\+ = lowest speed

The Multipette Xstream as well as the instrument by manufacturer A were able to dispense glycerol of a viscosity of 836 mPa*s up to a tip size of 2.5 ml at the highest speed setting. Greater dispensing volumes required speed reduction. Instruments by manufacturers B and C could be used at highest speed with tip sizes of 10 ml and 25 ml, but only when the viscosity is reduced considerably (Table 3).
While maximum dispensable viscosity was greatly reduced above 5 ml for instruments by manufacturers B and C, both the Multipette Xstream and a hand dispenser by manufacturer A were able to dispense comparatively viscous solutions at the lowest speed setting. As a rule, the factors viscosity, tip size and dispensing volume work synergistically. The higher two of these factors are, the slower the recommended speed.

Conclusion

Air cushion pipettes are not suitable for precise dispensing of viscous liquids. Even consideration of special pipetting techniques will not entirely offset the effects of high viscosity, and dispensing results will fall outside the error tolerances. The larger the air cushion (e.g. 100 µl dispensed using a 1000 µl tip), the greater the error. In contrast, positive displacement instruments such as the Multipette plus or the Multipette Xstream perform without error under these conditions when handled appropriately.

Furthermore, electronic dispensing instruments considerably reduce the force transferred to the user during dispensing of viscous liquids. However, the highest dispensable viscosity is dependent on tip size as well as dispensing volume: The greater the dispenser tip, the lower the dispensable viscosity. During dispensing of highly viscous liquids the piston speed is to be reduced. Alternatively, a manual dispensing instrument may be used, such as the Multipette plus.

Literature

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Ordering information

Product name	Order no. international	Order no. North America
Multipette plus/Repeater plus	4981 000.019	022260201
Multipette Xstream/Repeater Xstream	4986 000.025	022460811
Reference fix 100 µl	4900 000.133	022471155
Research 100–1000 µl	3111 000.165	022472003
ep T.I.P.S. 2–200 µl (Standard)	0030 000.870	022492039
ep T.I.P.S. 50–1000 µl (Standard)	0030 000.019	022492055
Assorted Combitips plus (Standard)	0030 069.285	022266624

Combitips plus	Order no. international	Order no. North America	Order no. international	Order no. North America
	Standard (100 each)		Eppendorf Biopur (individually wrapped, 100 each)	
0.1 ml	0030 069.200	022265954	0030 069.404	022496000
0.2 ml	0030 069.218	022266004	0030 069.412	022496026
0.5 ml	0030 069.226	022266101	0030 069.420	022496042
1.0 ml	0030 069.234	022266209	0030 069.439	022496069
2.5 ml	0030 069.242	022266306	0030 069.447	022496085
5.0 ml	0030 069.250	022266403	0030 069.455	022496107
10 ml	0030 069.269	022266501	0030 069.463	022496123
25 ml	0030 069.293	022266551	0030 069.390	022496131
50 ml	0030 069.277	022266608	0030 069.471	022496140

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