Pipette Operation Guide

For accurate dispensing with pipettes – Rev.3

2015/10/15

R&D Division 5
A&D Company, Limited

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For Accurate Dispensing With Pipettes

—Dispensing Specialty Liquids with MPA Series Electronic Pipettes—

Foreword

Accuracy management of pipettes is performed using purified water; however in the actual place of use it is not uncommon to dispense viscous liquids, volatile liquids, or even strong acids. This document summarizes pipette dispensing methods for such liquids and the MPA Series' resistance to organic solvents, etc.

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1. Dispensing viscous liquids

1-1 Methods for dispensing viscous liquids

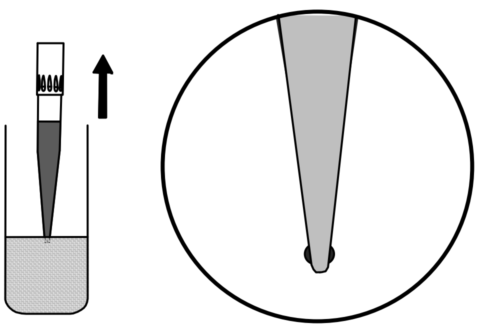
In general, viscous liquids are likely to stick to the inside of the pipette's tip, making accurate dispensing (aspirating/discharging) difficult.
If you wish to accurately dispense liquids with high viscosity, the methods below are recommended.

(1) When aspirating the liquid, do not dip the tip deeply beyond the surface of the liquid
(2) When aspirating the liquid, wait a few seconds after the aspiration action is completed with the tip left in the liquid
(3) Use a tip with a large tip end diameter (cut off the end of the tip, etc.)
(4) Reduce speed when discharging
(5) Use Reverse mode

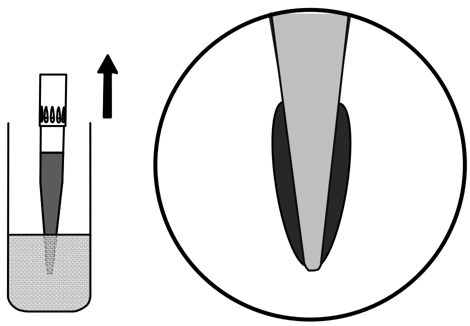
1-2 Detailed Explanation

(1) When aspirating the liquid, do not dip the tip deeply beyond the surface of the liquid

If liquid sticks to the periphery of the tip, it may be dispensed when discharging which could lead to errors in dispensing amounts.
By minimizing the depth that the tip is submerged in a liquid when aspirating, the amount of liquid sticking to the periphery of the tip can be reduced and errors in dispensing amounts minimized.



Liquid stuck to the tip



Liquid stuck to the tip

(2) When aspirating the liquid, wait a few seconds after the aspiration action is completed with the tip left in the liquid

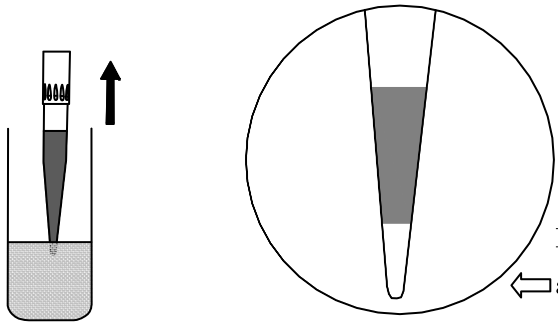
When aspirating liquids, as the piston rises, the air in the space between the piston and the liquid loses pressure and the liquid is aspirated into the tip.
If the liquid has a high viscosity, the liquid will continue to be slowly aspirated even after the piston has stopped until the low pressure air has reached equilibrium and the liquid

level inside the tip will rise.

In order to accurately aspirate the specified amount, keep the end of the tip in the liquid for a few seconds until the liquid inside the tip stops rising.

If air enters the tip immediately after the tip is removed from the liquid, please keep the tip submerged in the liquid for a short while longer as the air within the tip has not yet reached equilibrium.

[Example: Insufficient wait time]



Low pressure air causes the liquid to be aspirated even after the piston has stopped

(3) <u>Use a tip with a large tip end diameter (cut off the end of the tip, etc.)</u>

The movement of liquid when aspirating or discharging encounters maximum resistance in the small-diameter section at the end of the tip.

As this resistance will increase with high viscosity liquids, widening the diameter of the tip end (cutting the end or using wide diameter tips) will reduce resistance when aspirating and discharging and make more accurate dispensing possible.

In this case, there will be a tendency for the dispensed amount to increase due to liquid sticking to the wider tip end more easily. (Refer to Graph 1)

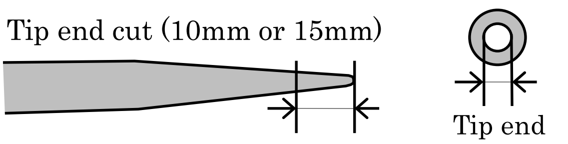
[For the MPA-200]

Dispensed amount difference with cut A&D tip

	10mm cut	15mm cut
Tip end diameter	Ø1.36mm	Ø1.88mm
200 uL	1.1 uL	1.6 uL
10 uL	0.6 uL	1.6 uL

* Diameter of standard non-cut tip is 0.54mm
* Purified water was dispensed
* Difference in dispensing amounts
= (amount for cut tip) - (amount for standard tip)

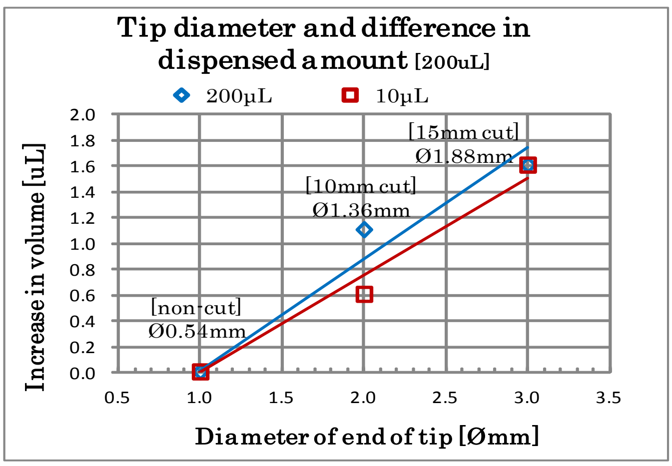
Tip end cut (10mm or 15mm)



Tip end diameter

* Measurement of 200 µL and 10 µL using the MPA-200 with standard A&D tip for 200 µL

[Graph 1]

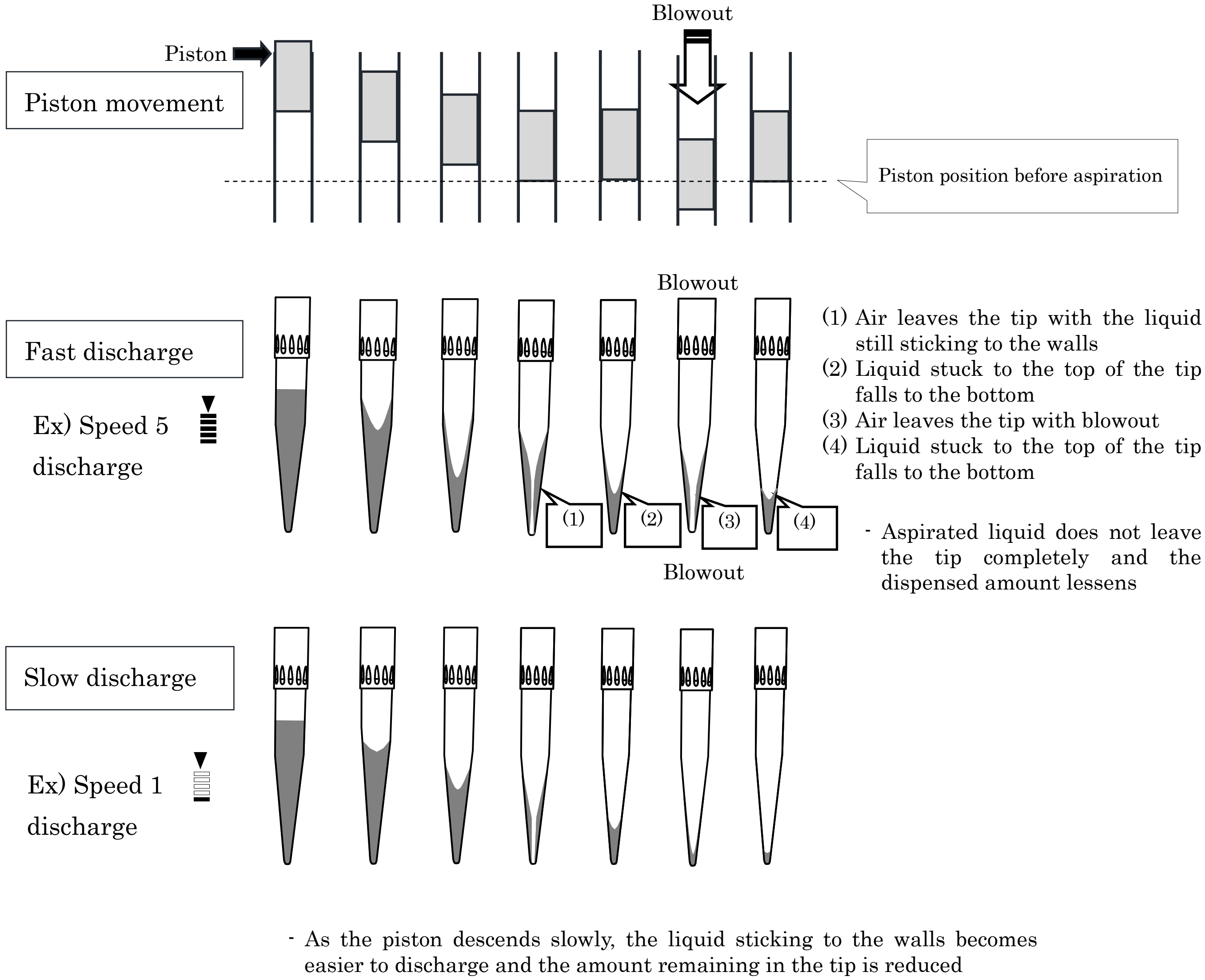


(4) Reduce speed when discharging

Viscous liquids tend to stick to tips easily. When an aspirated high viscosity liquid is later discharged, the portion of the liquid sticking to the internal walls of the tip descends very slowly. The discharging action involves lowering the piston which compresses the air and discharges the liquid from the tip.

Therefore, if the discharging speed is fast, the air will be expelled before the liquid sticking to the inner walls sinks to the bottom of the tip, preventing the liquid inside the tip from being completely discharged and reducing the amount dispensed.

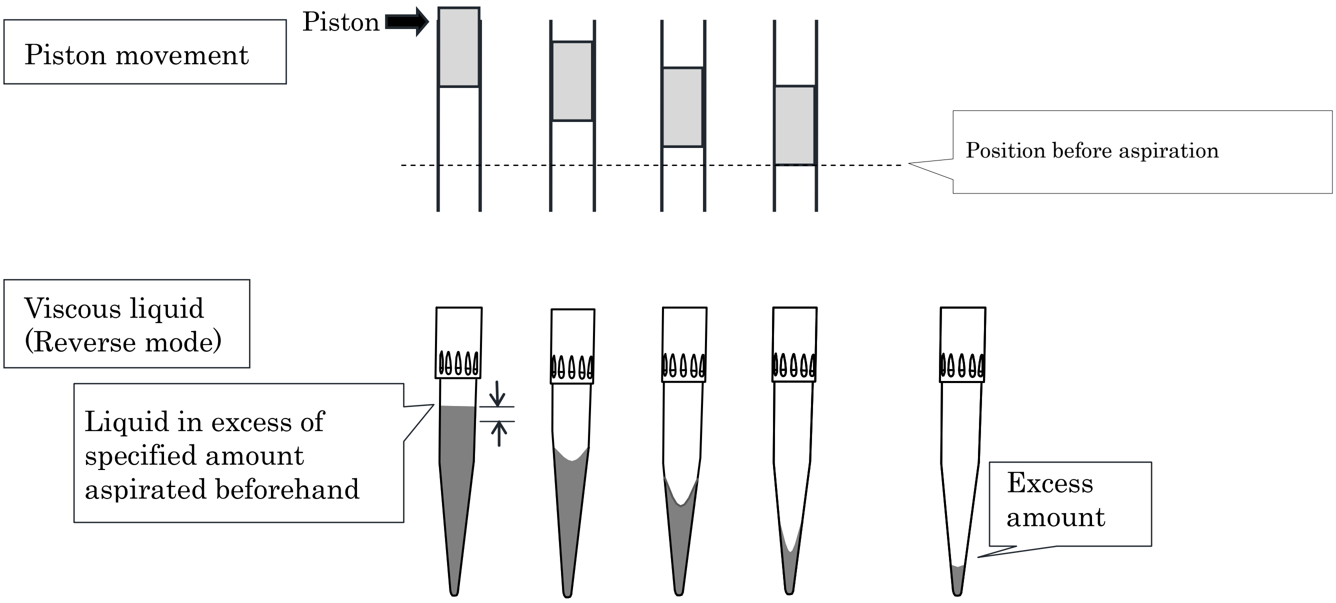
By setting the discharging speed to a slow rate, the compression of the air in the tip at the time of discharge will occur more slowly and the liquid in the tip will have plenty of time to sink to the bottom ensuring air is less likely to be discharged.



(5) Use Reverse mode

This dispensing method aspirates more liquid than the specified amount but only discharges the specified amount.

By aspirating more liquid than is required, air is unlikely to be discharged even if the liquid sticks to the internal walls of the tip and the specified amount can be discharged easily.



1-3 Dispensing results for viscous liquids

The information below shows the dispensing results for liquids of differing viscosities (thickening agents/water solution with glycerin added) using the MPA-200. (Refer to Graph 2-4)

Each type of viscous liquid was dispensed by combinations of maximum or minimum aspiration/discharge speed and the standard blowout (*1) or reverse mode (*2) dispensing method.

Lowering the aspiration/discharge speed and using the reverse mode was found to allow accurate measurement of even high viscosity liquids. It was also learned that for a solution with a viscosity of around 20mPa.s made from water and a common thickening agent, experimenting with the dispensing method could lead to improved accuracy in dispensing.

*1. **Blowout**: Method involving aspiration of a specified amount of liquid and then discharging that entire amount.
In order to discharge all the liquid, the piston is thrust down further than its starting point when the liquid was aspirated.
(For a manual pipette, the action is pushing down to the second button.)

*2. **Reverse Mode**: Method which aspirates more than the specified amount, but only discharges the specified amount.

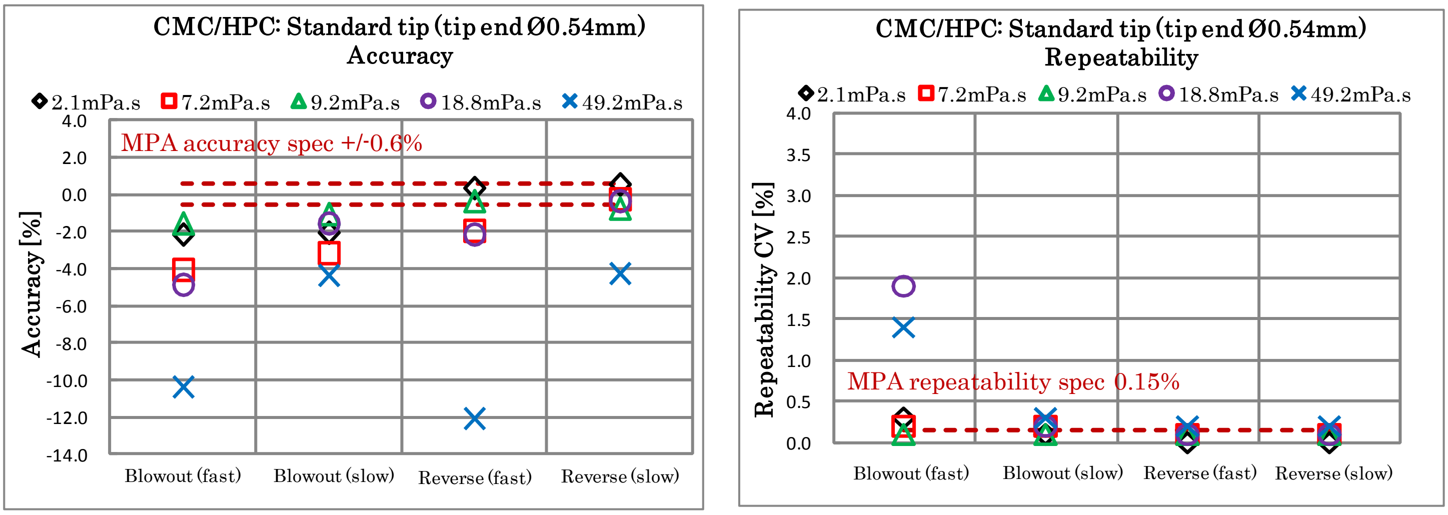
1) Dispensing results of thickening agents

Thickening agents: HPC (Hydroxypropylcellulose)

CMC(Carboxymethylcellulose sodium)

Thickening substance	Concentration	Viscosity
HPC2.0	2.0%	2.1mPa.s
HPC6.0-10.0	2.0%	7.2mPa.s
CMC	0.2%	9.2mPa.s
CMC	0.5%	18.8mPa.s
CMC	1.0%	49.2mPa.s

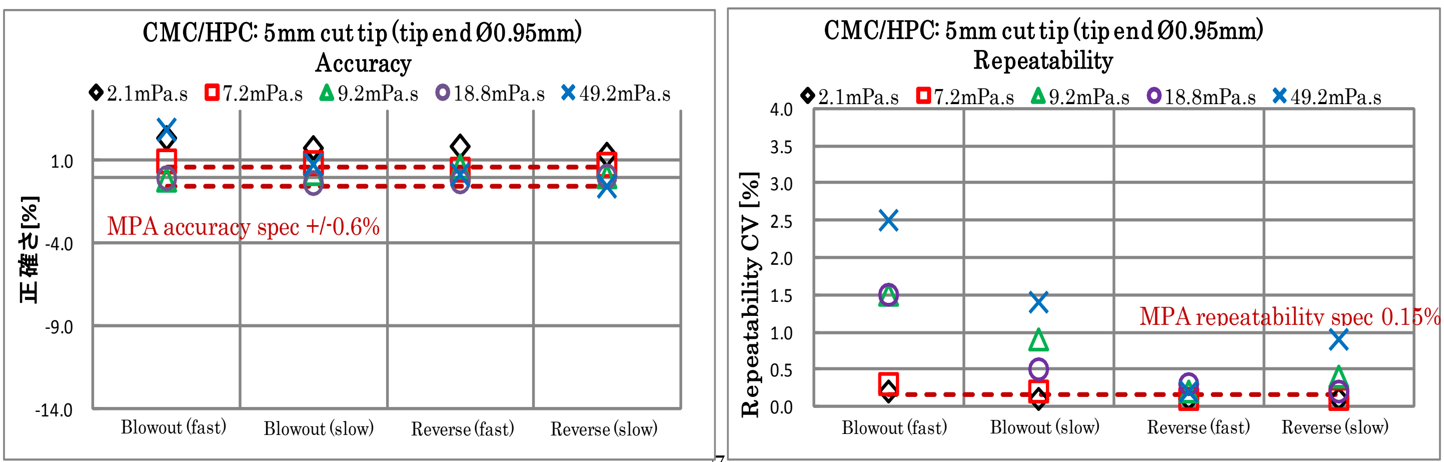
［Graph 2］ Dispensing 200 µL with the MPA-200



When viscosity levels reach around 50mPa.s, air is discharged before the liquid and the amount dispensed becomes less than the amount specified.

Even at around 50mPa.s, if the end of the tip was cut wider it became possible to reduce resistance when discharging and improve accuracy of dispensing amounts. In return, however, repeatability worsened. (Refer to Graph 3)

[Graph3] 200 µL dispensed with the MPA-200

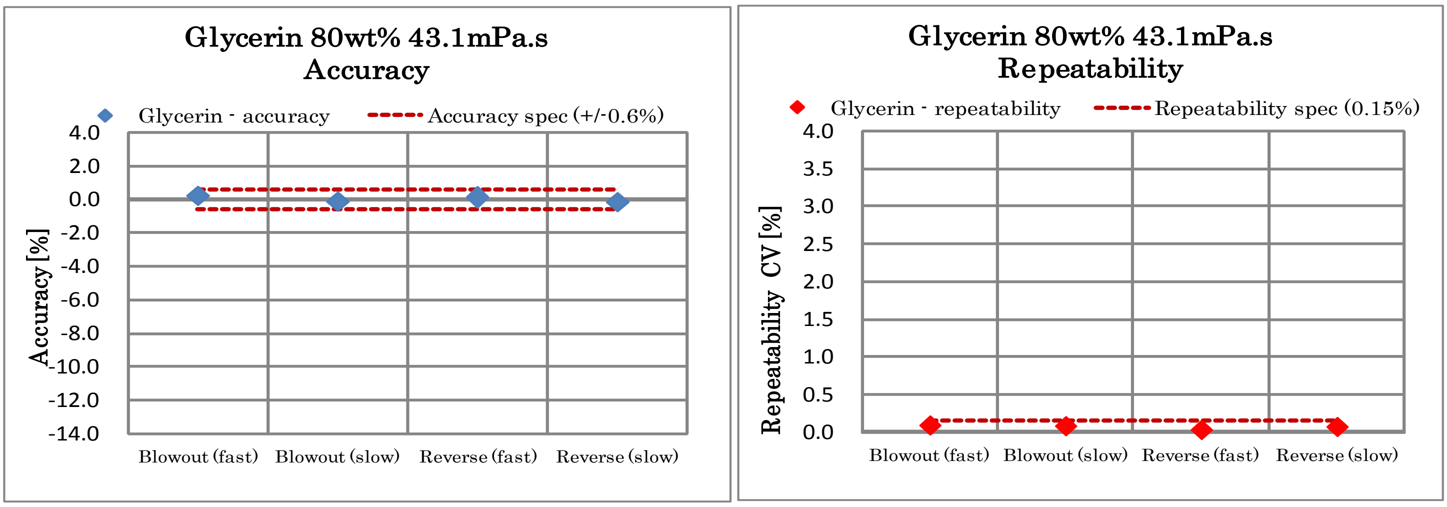


2) Dispensing results for glycerin solution

The glycerin solution (80% concentration, approximately 43.1mPa.s) had nearly the same viscosity as the thickening agent (CMC 1.0% water solution, approximately 49.2mPa.s) but had good dispensing accuracy.

This was judged to be a result of the glycerin solution being unlikely to stick to the tip (made from polypropylene) and having properties which made it unlikely to remain in the tip when discharged. (Refer to Graph 4)

[Graph 4] 200 µL dispensed with the MPA-200

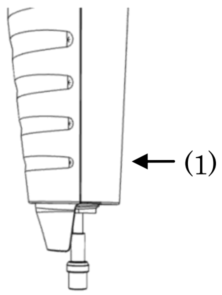
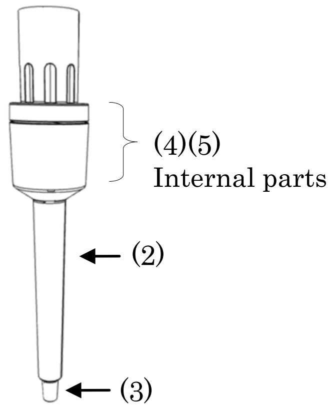
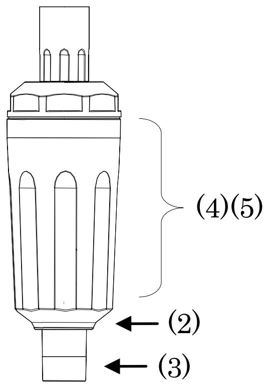


2. Dispensing volatile organic solvents

2-1 Resistance to organic solvents

The pipette materials are detailed below.

#	Name	[MPA-10/20/200/1200] Materials	[MPA-10000] Materials
(1)	Main unit	ABS (Acrylonitrile-butadiene-styrene)	ABS (Acrylonitrile-butadiene-styrene)
(2)	Tip ejector	PP+GF20% (Polypropylene with 20% fiberglass)	PP+GF20% (Polypropylene with 20% fiberglass)
(3)	Tip holder	PVDF (Polyvinylidene fluoride, fluorinated resin)	PVDF (Polyvinylidene fluoride, fluorinated resin)
(4)	Piston	SUS303 (Grease coating type)	
(5)	O-ring	NBR (Nitrile rubber)	NBR (Nitrile rubber)

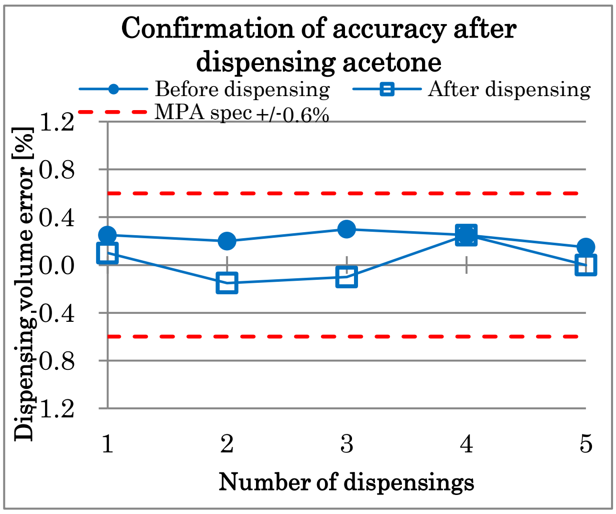
MPA Main unit	Lower part	
	[MPA-10/20/200/1200]	[MPA-10000]
		

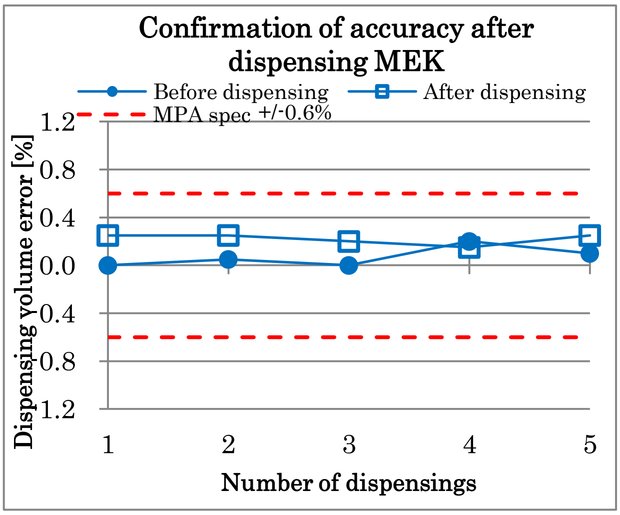
As aspiration and discharge of the liquid is performed using air as an intermediary, the tip is the only part that directly touches the liquid when handling organic solvents.
After aspirating, the vaporized volatile elements of the organic solvent reach the internal structure of the pipette.
The tip which is in direct contact with the liquid is made from PP (polypropylene), so it has resistance to common organic solvents and can also be easily replaced.
The parts of the pipette that are reached by the vapors emitted by the volatile elements of the organic solvent after aspiration are the tip holder, piston and O-ring. The tip holder is made from PVDF, a fluorinated resin, so it has exceptional chemical resistance; the piston and O-ring are coated in a fluorinated grease, so they have high chemical resistance. Accordingly, the MPA Series maintains a certain level of resistance even when dispensing organic solvents.

The parts that may directly touch solvents or are exposed to gases due to their vaporization can easily be replaced altogether as a lower part unit at user level.

Graph 5 shows the results of tests of whether the dispensing of acetone and MEK (methyl ethyl ketone) has any influence on pipette accuracy after performing dispensing of these substances 200 times.

[Graph 5] 200 μL dispensed with the MPA-200

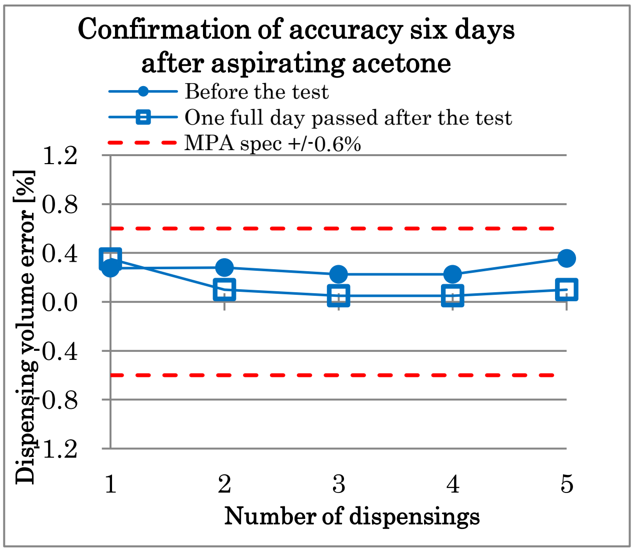


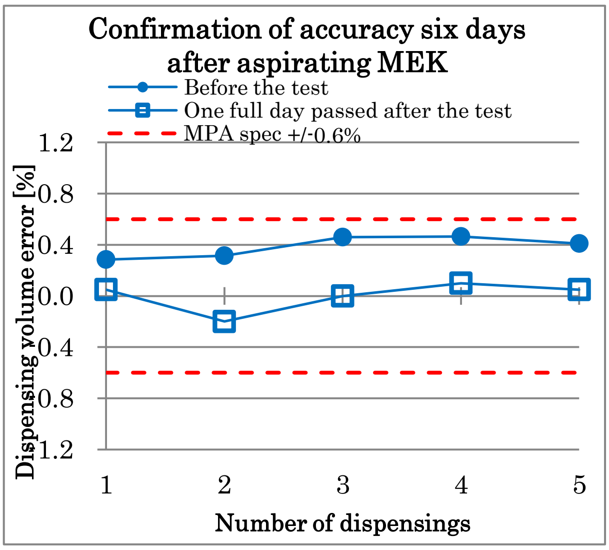


* Accuracy was tested half a day to a full day after dispensing the organic solvents

Graph 6 shows the results of testing the pipette's accuracy by dispensing purified water after aspirating acetone and MEK and leaving the pipette in that condition for six days, hanging it on its pipette stand.

[Graph 6] 200 μL dispensed with the MPA-200





* Accuracy was tested one day after discharging the solvents from inside the tip

* The acetone completely vaporized three days after the start of the experiment and the MEK

completely vaporized two days after for the MPA-10000 experiment. For this reason pipettes were left out for three days and accuracy was tested one day after that.

These results show that even using strong organic solvents such as acetone or MEK do not reduce the accuracy of the MPA Series in the short-term.
In a similar experiment with the MPA-10000 there was no influence on the accuracy of the pipette.

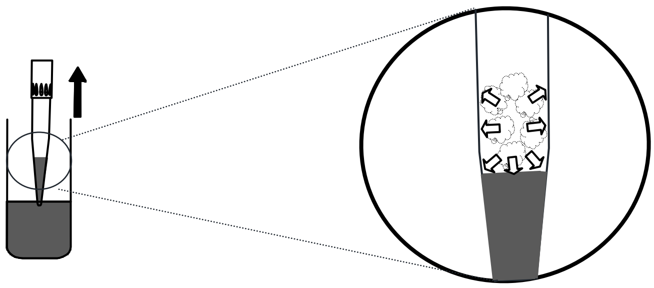
2-2 Methods for dispensing organic solvents

When dispensing volatile liquids, the volatility of the liquid aspirated may have an influence on the volume of the air inside the pipette. If you wish to increase accuracy when dispensing volatile liquids, please thoroughly pre-rinse (*1) immediately before dispensing (at least five times).
*1 Using the liquid to be dispensed, repeat aspiration and discharge several times.

2-3 Detailed explanation

Aspiration and discharge of the liquid is performed using the air pressure between the internal piston in the pipette and the liquid as an intermediary. If the liquid becomes volatile (vaporizes) while it is being aspirated or while it is in the pipette, the air pressure within the pipette will rise and that air pressure will push the liquid down meaning it may become impossible to aspirate specified amounts of liquid. (Refer to Illustration 1)
Accordingly, when aspirating volatile liquids it has been found that the liquid may sometimes drip down from the end of the tip.
Pre-rinsing the pipette several times immediately before dispensing will mean that the space inside the pipette will become saturated, preventing vaporization of the aspirated liquid and allowing accurate dispensing.

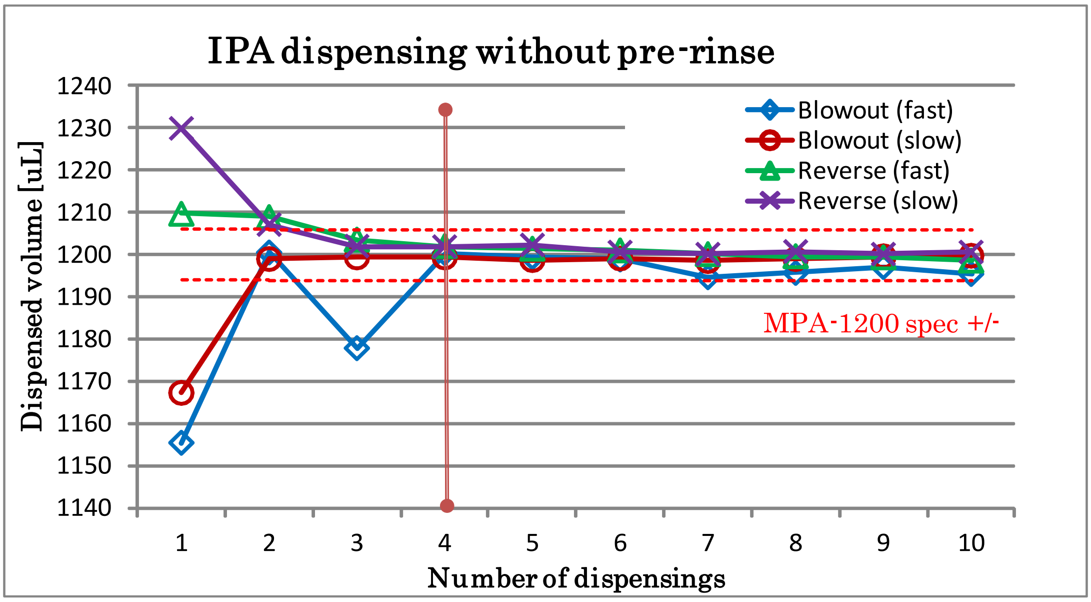
[Illustration 1]



2-4 Dispensing results for organic solvents

Graph 7 shows the results for repeated dispensing of volatile IPA (isopropyl alcohol) without pre-rinsing the pipette beforehand. Dispensed volumes were not stable the first few times, but from the fourth dispensing onwards it was found that dispensing results became stable. Accordingly, it appears that pre-rinse must be performed at least three times before dispensing to ensure accurate dispensing amounts.

[Graph 7] Dispensing 1200 µL with the MPA-1200



Graph 8 shows the results for repeated dispensing of volatile acetone and MEK (methyl ethyl ketone) without pre-rinsing the pipette beforehand.

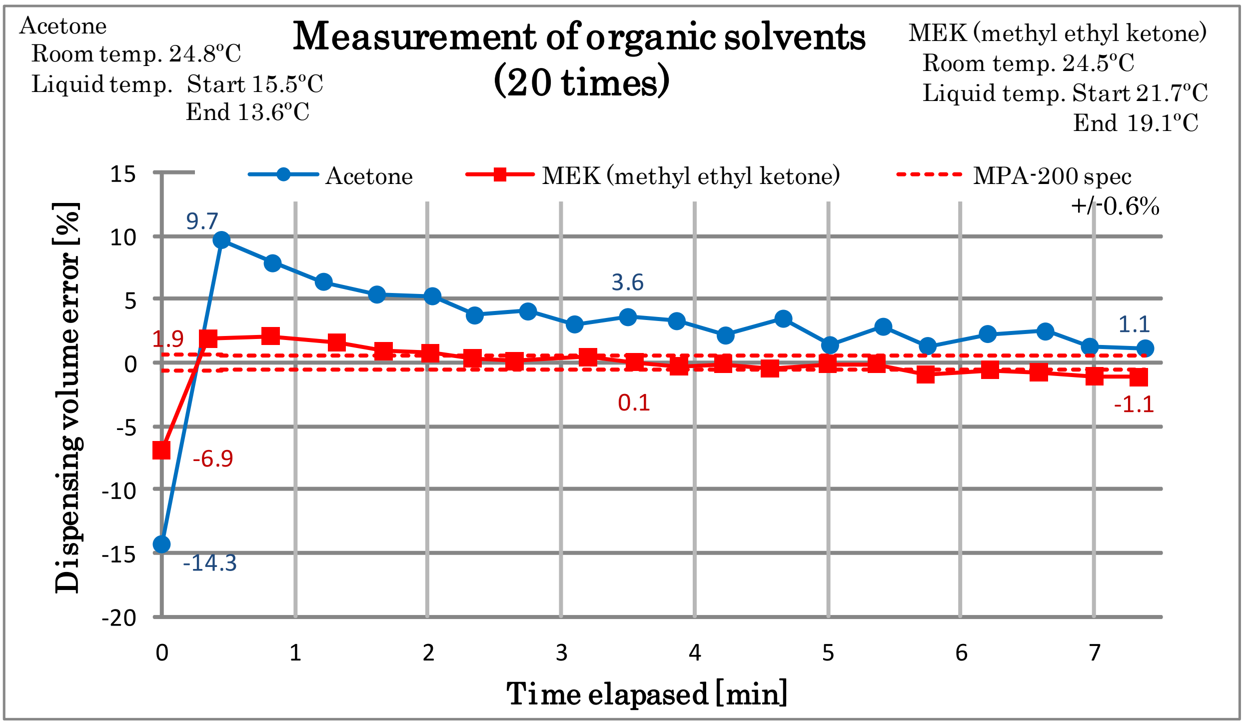
Acetone has an evaporation rate ten times faster than water and MEK has a rate four times faster. The acetone had a temperature 10°C lower than the ambient temperature and the MEK was 5°C lower than the ambient temperature.

Both the acetone and MEK initially were dispensed at smaller volumes than the specified amounts, but this later increased and in the end volumes approached the amounts that were specified (200 µL).

This can be seen as a tendency for the first dispensing to be smaller than specified as a result of the air pressure inside the pipette increasing due to the volatility of the solvent. It can be inferred that the tendency for the dispensing amount to increase from the second dispensing onwards is from the pressure decreasing with the tip cooling due to the volatility of the solvent.

In any case, it is clear that when wishing to accurately dispense a liquid with high volatility at a temperature which differs from the ambient one, it is necessary to pre-rinse the pipette a sufficient number of times immediately before dispensing.

[Graph 8] 200 µL dispensed with the MPA-200



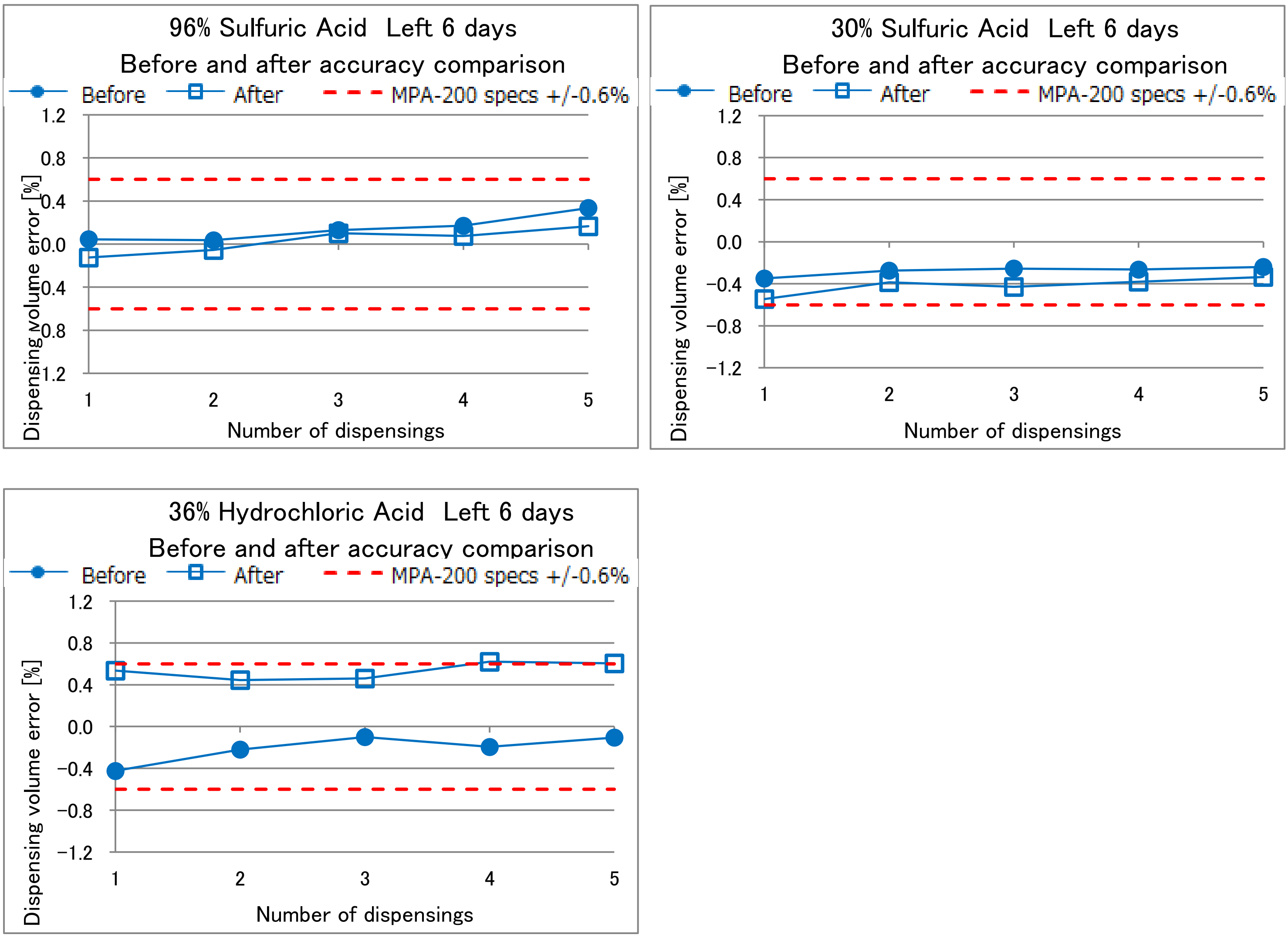
3. Dispensing acids

3-1 Resistance to acids

Below the MPA-200 was used to aspirate acid into the tip and left in that condition on a pipette stand for 6 days. The pipette's accuracy was checked with purified water before and after the 6 days. (Refer to graph 9)

- 96% Sulfuric acid (96% concentration undiluted solution, viscosity 23 mPa.s)
- 30% Sulfuric acid (96% concentration solution diluted with purified water)
- 36% Hydrochloric acid (35-37% test solution)

[Graph 9] 200 μL dispensed with the MPA-200



For the sulfuric acid (96%, 30%) there was no observed deterioration of the main body or effect on the accuracy of measurement.

The lower part (tip holder) and the piston of the main body turned yellow during the hydrochloric acid resistance test; however, there were no accuracy problems.

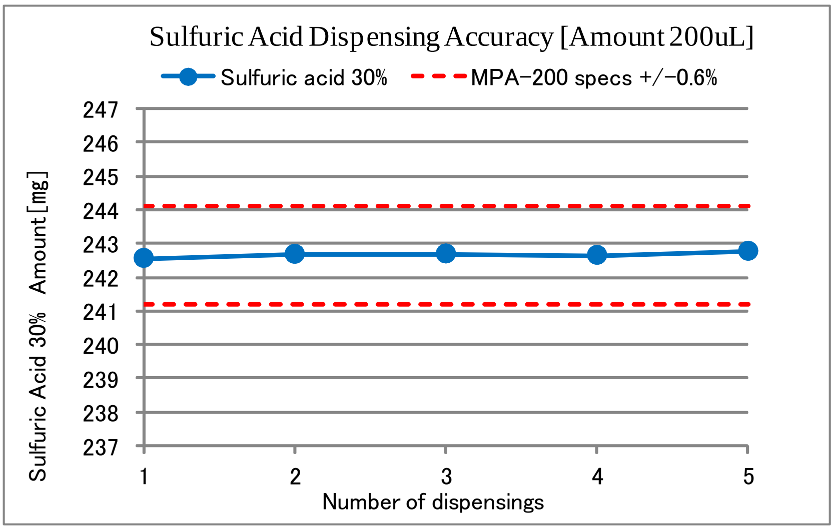
3-2 Dispensing results for acids

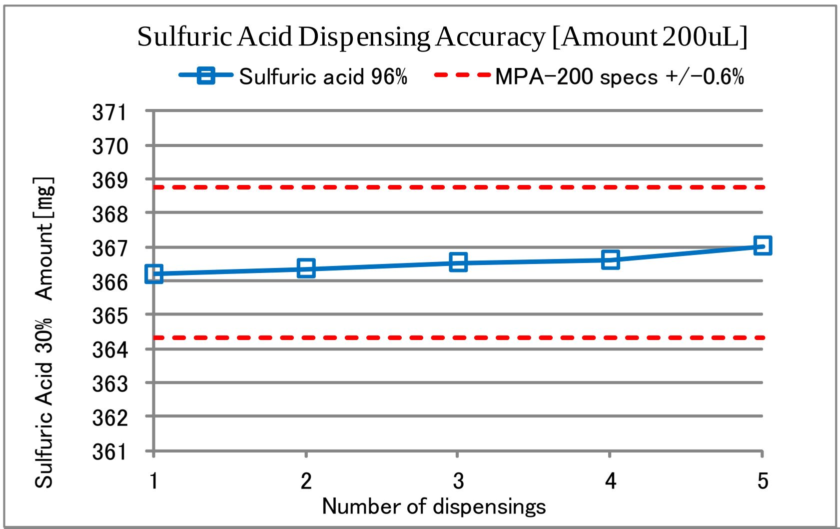
Graph 10 shows the measured results for dispensed amounts of acid.

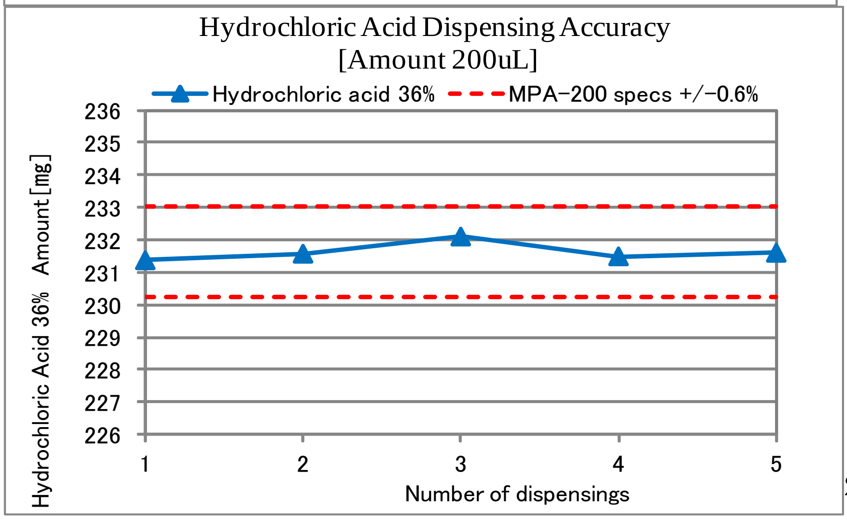
The slowest aspirating/discharging speed (MPA-200 Speed 1) was set for the viscous 96% sulfuric acid and the device was sufficiently pre-rinsed for dispensing the volatile hydrochloric acid.

We confirmed it was possible to dispense a fixed amount with no variation.

[Graph 10] 200 μL dispensed with the MPA-200







4. Tip compatibility

4-1 Comparison of dispensed amounts between standard tips and filter tips

Graph 12 shows the results of tests checking if there is a difference in dispensing amounts between tips with filters attached and those without.

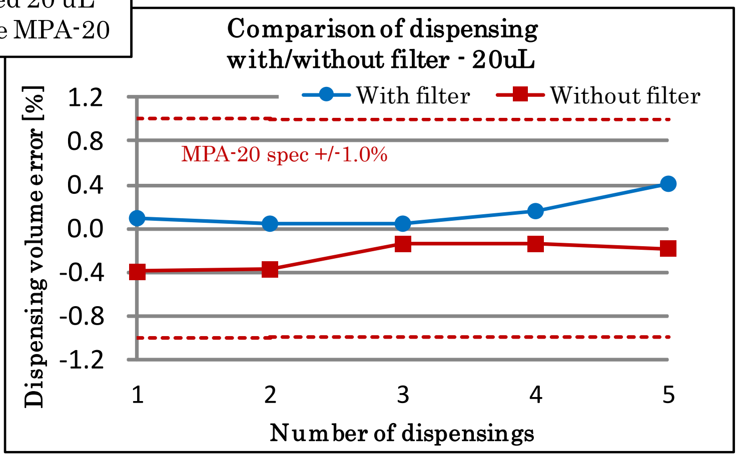
The results showed that the dispensing amount did not change depending on the filter being there.

Pipette aspiration and discharge is done through the intermediary of the air between the piston and the liquid.

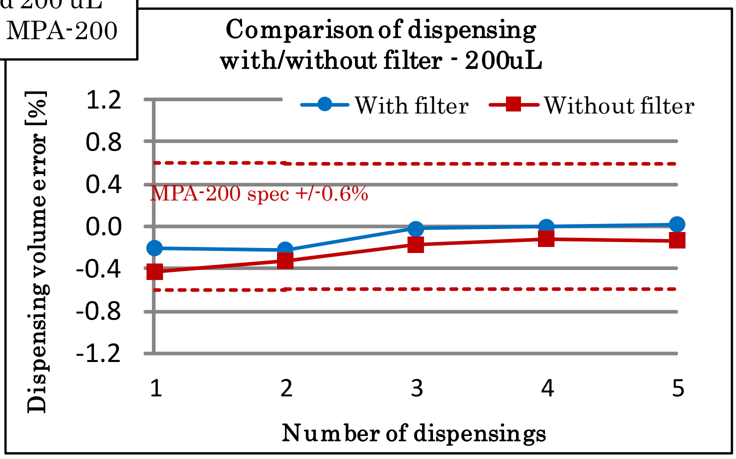
Compared to the resistance of a liquid passing through the small hole at the end of a tip, the resistance of air (1/50 the viscosity of water) going through a tip's filter can be considered tiny.

[Graph 12]

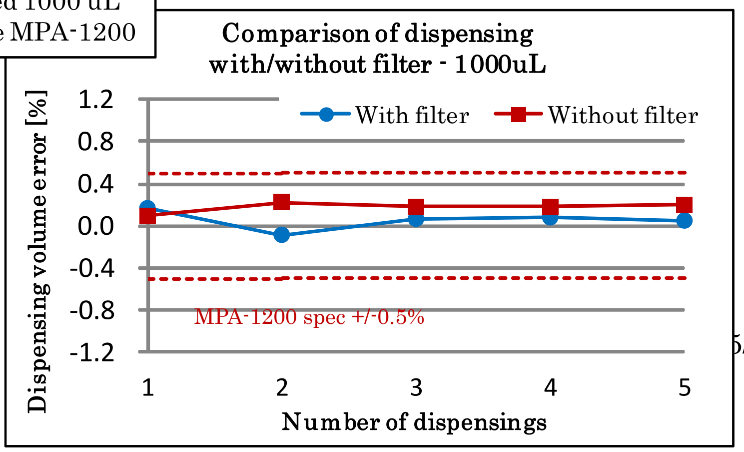
Dispensed 20 uL using the MPA-20



Dispensed 200 uL using the MPA-200



Dispensed 1000 uL using the MPA-1200



4-2 Compatible tips for the MPA series

The following table shows tip compatibility for the MPA series.

Pipette	Tip maker	Model number	Compatibility	Remarks
MPA−10/20	Gilson	D10	Compatible	
		DL10	Incompatible	Not possible to affix tip
	Eppendorf	epT.I.P.S. Standard	Compatible	
	Rainin	RC−10	Compatible	
	Biohit	790014	Compatible	
	Thermo	Flex10	Compatible	
	BRAND	702504	Compatible	
	Nichiryo	BMT−SS	Compatible	
	QSP	101	Compatible	
		114	Incompatible	Not possible to affix tip
	Watson	207C	Compatible	
	Nippon Genetics	37660	Compatible	
		10310	Compatible	
	Labcon	1038	Compatible	
MPA−200	Gilson	D200	Compatible	
	Eppendorf	epT.I.P.S. Standard	Compatible	
	Rainin	RC−250	Compatible	
	Biohit	790204	Compatible	
	Thermo	Flex200	Compatible	
	BRAND	702516	Compatible	
	Nichiryo	BMT−SE	Compatible	It is necessary to adjust the height of the tip ejector
	QSP	110−NEW	Compatible	
		110−N	Compatible	
		110−B	Compatible	
	Watson	705Y	Compatible	
	Nippon Genetics	FG−301RS	Compatible	
		FG−302	Compatible	
		10340	Compatible	
	Labcon	1093	Compatible	
MPA−1200	Gilson	D1000	Caution	At aspiration of 1200uL, the tip holder becomes wetted Use is possible at aspiration of 1000uL
	Eppendorf	epT.I.P.S. Standard	Compatible	
	Rainin	RC−1000	Compatible	
	Biohit	791005	Compatible	
	Thermo	Flex1200	Compatible	
	BRAND	702521	Compatible	
	Nichiryo	BMT−L	Compatible	
	QSP	111	Compatible	
	Watson	706B	Compatible	
	Nippon Genetics	34760	Compatible	
	Labcon	1045	Compatible	
	Greiner Bio−One	750251	Compatible	
MPA−10000	Gilson	D10mL	Compatible	The tip requires manual detachment
	Eppendorf	epT.I.P.S. Standard	Compatible	
	Rainin	17001122	Compatible	The tip requires manual detachment
	Thermo	Finntip 9402150	Incompatible	Not possible to affix tip
	BRAND	702603	Compatible	The tip requires manual detachment
	Nichiryo	00−BMT2−Z	Incompatible	Not possible to affix tip
	QSP	097	Compatible	The tip requires manual detachment
	Watson	409C	Compatible	The tip requires manual detachment
	Labcon	442C6−442	Compatible	
	Axygen	1910−01	Compatible	The tip requires manual detachment

Compatibility

Compatible	No problems with affixing tip, ejecting, or aspirating and dispensing The tip's accuracy has been confirmed to within +/−1.5% (at the maximum volume) of the levels of A&D's standard tips (With the MPA's volume calibration functionality, it is possible to increase accuracy further)
Caution	Limits to volumes for use
Incompatible	Not possible to affix tip

5. Appendix of measurement data

5-1 Dispensing soy sauce

Dispensing measurements were compared for five different types of soy sauce.

The pipette was set to reverse mode and also the aspirate/discharge speed was set to the lowest setting (speed 1) as soy sauce has a higher viscosity compared to purified water.

Graph 11 shows the results of dispensing 1000 µL of purified water and each of the five types of soy sauce 10 times.

Samples A, B and C show a larger amount dispensed on the first time and gradually trend towards the set amount after repeated dispensing.

Reasons for this are:

- There is a larger amount of liquid in the tip from setting the device to reverse mode.
- The vaporization of the liquid effected the movement of the piston while discharging and caused a greater amount of liquid to discharge after the first aspiration since the pipette was not pre-rinsed.
- Repeated dispensing had the effect of pre-rinsing the pipette, reducing the vaporization of the liquid allowing for more accurate values.

  Samples D and E dispensed less on the first time, reached the highest amount on the second time and gradually trend towards the set amount after repeated dispensing.

Reasons for this are:

- The liquid is highly viscous and often remains stuck to the inside wall of the tip. This causes air to discharge before the liquid resulting in a smaller amount on the first dispensing.
- Air can no longer discharge before liquid on the second and subsequent dispensing since the liquid already is stuck to the inner wall of the tip.
- In a way similar to samples A, B and C repeated dispensing has the effect of pre-rinsing the pipette, reducing the vaporization of the liquid allowing for more accurate values.

	Water	Sample A	Sample B	Sample C	Sample D	Sample E
Viscosity [mPa・s]	0.94	2.66	2.59	2.37	17.0	16.0
Density [g/cm ^{2}]	0.9968	1.1644	1.1686	1.1082	1.2705	1.2258

[Graph 11] 1000 µL dispensed with the MPA-1200