AN049: PIPETTING – DISC PUMP APPLICATION NOTE

1. INTRODUCTION

The disc pumps are a silent, high-performance piezoelectric micropump.

Owing to its operating mechanism, the disc pumps can be controlled with unmatched precision, yet at the same time respond to full-scale set point changes in a matter of a few milliseconds. The compact form factor means it can be tightly integrated into products, increasing portability.

Figure 1: A piezoelectric disc pump

1.1.About this Application Note

This Application Note introduces a novel pipetting scheme—known as the Volume Control Module (VCM)—that takes advantage of the high-precision pressure control provided by disc pump. The note covers:

- The basic design and operation of a VCM.
- Guidance on optimising VCM performance, including key considerations and pitfalls to avoid.
- Data from The Lee Company's own prototype systems.
- Possible design inclusions for increased utility.
- Useful equations.

Our intention is that this note provides a helpful starting point for customers considering implementing this scheme in their own products.

Please note that The Lee Company does not design/manufacture pipetting modules.

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3. THE VOLUME CONTROL MODULE (VCM)

3.1.Introduction to the VCM

When dosing controlled liquid volumes, a syringe pump or air displacement pipette are often used. These technologies offer excellent precision but are often large and expensive. An alternative approach to this method is proposed.

Due to the high responsiveness and precise control of disc pump, a method based on pressure control can be used. This is opposed to the “volume control” approach used by syringe pumps (i.e. by plunger position).

Pressure control is already widely used in dosing: the predominant approach, “pressure-time”, involves applying a known pressure for a fixed time to a body of fluid. This has the disadvantage that it is sensitive to timings, viscosity of the fluid, and the pressure difference across fluid.

The system proposed herein also uses pressure control; however, it works more similarly to an air displacement pipette, by displacing a controlled amount of gas from the pipette tip into a reservoir. The amount of gas is regulated by controlling the pressure increase in the reservoir. In turn, the amount of fluid aspirated/dispensed can be controlled. This system is called a Volume Control Module (VCM).

The Lee Company has built a prototype VCM system as a proof-of-concept, enabling us to explore the capability of this approach, key design considerations and pitfalls to avoid. Section 4 provides a detailed results collected with a prototype VCM. In summary, the system delivered a CV of less than 1% over the dosing range explored, from approximately 14 µL to 387 µL.

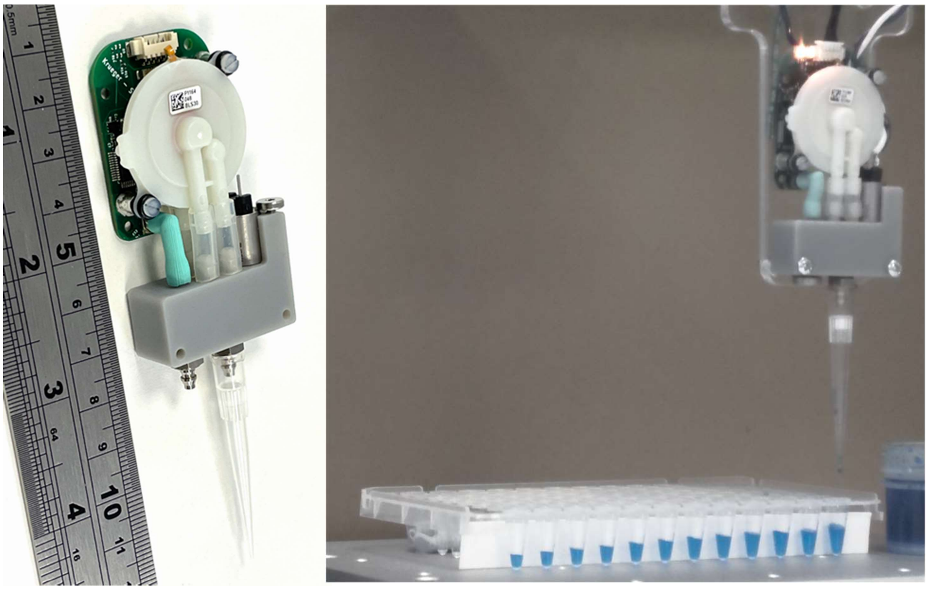


Figure 2: Left - Prototype VCM system, using The Lee Company Smart Pump Module and a custom pipetting manifold block. Right - Using the same prototype to dispense aliquots of increasing volume into successive wells of a 96-well plate.

3.2.Architecture

In its most basic form, the VCM comprises a pump driven into a reservoir (left-hand panel in Figure 3). As gas is pumped into the reservoir the pressure increases by an amount proportional to the amount of gas moved. Therefore, if the pump inlet is connected to a closed system, a known amount of gas can be removed from the system. This known amount of gas can be used to move a known amount of liquid, in the same manner as a conventional air displacement pipette. Unlike pressure-time dispensing, this setup does not require precise timing and is less dependent on gravity effects and fluid viscosity.

3.2.1. The Feedback Orifice

There is a problem with this simplified VCM system, however: disc pump is unidirectional, in that gas only flows freely through it in one direction; as a result, fluid can be aspirated but not dispensed. This can be solved by adding a feedback orifice between the reservoir and the system (as can be seen in the right-hand panel of Figure 3). This allows pressure in the reservoir to dissipate through the orifice, in turn enabling aspirated fluid to be dispensed.

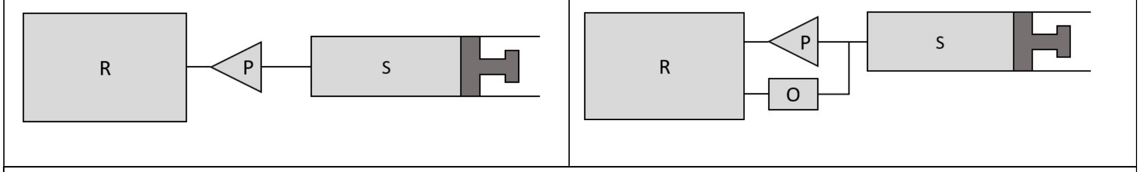


Figure 3: On the left a diagram of the most basic possible VCM and on the right a feedback orifice has been added to allow for dispense as well as allowing correction to overshoot of the pressure.

P is the pump showing pumping direction.

R is the reservoir, with volume V_r, and pressure rise of ΔP_r.

S is the system being controlled, with a volume that changes by V_{asp}

O is the orifice, with flow restriction strength, Lohms, L.

Note that when using a feedback orifice, the pump must run continuously to maintain the desired pressure rise in the reservoir, as gas will escape through the orifice whilst the reservoir is pressurised. This in itself is not an issue, but does place importance on the correct selection of system components (e.g. pump, reservoir and orifice) for good operation. Selection of these components is considered in §**Error! Reference source not found.**

There are additional benefits to having a feedback orifice. First, if the pressure setpoint is overshot, the orifice allows the pressure to dissipate so that the overshoot can be corrected. Second, orifices enable the full-scale pressure range of the system to be adjusted to suit the application requirements. This can effectively provide greater pressure control precision where the required range is smaller (see Figure 4).

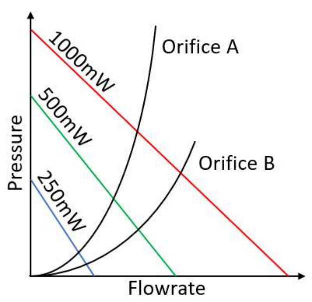


Figure 4: A schematic representation of a pressure vs. flow map, showing the intersection of orifice responses with the load lines of a pump at different drive powers (NB: not real data).

In this case, orifice A is more restrictive than orifice B and as such exhibits higher differential pressure for the same air flow rate – and therefore higher differential pressure for the same pump drive power. Note that pump load lines are not fixed and will vary with factors such as operating temperature, pump age, etc. Therefore open loop control of the pump should not be used as the pressure output at a given power is liable to vary.

3.3. Calculating the Volume of Fluid Aspirated

The volume aspirated by the system *S* can be calculated by using the Ideal Gas Law, $PV = nRT$, providing that the number of particles is kept constant (i.e. there are no leaks in the system). Therefore,

$$\Delta n_r = -\Delta n_s$$

Where *n* is the number of particles and *r* and *s* are reservoir and system, respectively. The result is that for a given aspiration pressure ΔP_r the volume aspirated is:

$$V_{asp} = -\Delta V_s = \frac{T_s}{T_r P_s} \cdot V_r \Delta P_r \left[= \frac{V_r \Delta P_r}{P_s} \textbf{ if } T_s = T_r \right]$$

Therefore the volume aspirated is linear with the aspiration pressure. Note that where the temperature of the reservoir and the system are not the same, the volume aspirated will also depend on T_r/T_s. However, this factor has a modest effect: a 3°C temperature difference results in only 1% change in volume aspirated, and therefore may only be a concern for high-precision applications.

3.4. Pre-charging the VCM reservoir to enable complete dispense

To ensure complete dispense, and to enable the blow-off functionality often used in pipetting workflows, it is helpful to pre-charge the VCM reservoir. To achieve this, before lowering the pipette tip into the fluid, run the pump and control the reservoir to a set pressure. This provides an excess of stored air that enables complete dispense.

3.5.VCM Pipetting process steps

Figure 5: VCM pipetting process steps

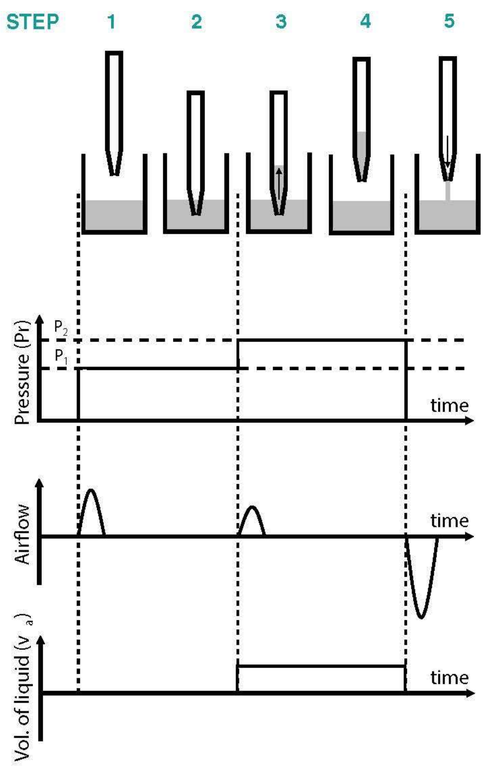
Step 1: With the tip above the fluid, the pump is turned on. The flow restriction created by the orifice causes the pressure in Vr to rise. The pump drive power is controlled so that a target pressure of P1 is reached in Vr. This elevated pressure provides an excess of stored air to ensure full and complete dispensing in step 5.

Step 2: The pipette tip is lowered into the fluid.

Step 3: The pump drive power is increased so that a higher target pressure, P2, is reached in Vr. An additional discrete quantity of air, proportionate to (P2-P1), is displaced from the tip into the system. The air pressure in the tip reduces, drawing fluid into the tip. The exceptional pressure regulation offered by Disc Pump in turn provides high-precision control over the quantity of fluid aspirated, Va.

Step 4: The pipette tip is withdrawn from the fluid.

Step 5: The pump power is reduced. The pressure in Vr discharges through the orifice into the tip. This results in fluid dispense from the tip. Dispense speed is controlled by the rate that the pump power (and therefore the pressure in Vr) is reduced - turning the pump off completely will result in the fastest dispense speed.



3.6. Component Selection

3.6.1. Reservoir and Pressure sensor selection

Using the equation from **Error! Reference source not found.**, Figure 6 shows how the liquid volume aspirated varies with reservoir volume and aspiration pressure.

Reservoir Volume	Liquid volume aspirated for given aspiration pressure					
	2 mBar	5 mBar	10 mBar	20 mBar	50 mBar	100 mBar
1 cc	2 µL	5 µL	10 µL	20 µL	50 µL	100 µL
2 cc	4 µL	10 µL	20 µL	40 µL	100 µL	200 µL
5 cc	10 µL	25 µL	50 µL	100 µL	250 µL	500 µL
10 cc	20 µL	50 µL	100 µL	200 µL	500 µL	1 mL
20 cc	40 µL	100 µL	200 µL	400 µL	1 mL	2 mL
50 cc	100 µL	250 µL	500 µL	1 mL	2.5 mL	5 mL
100 cc	200 µL	500 µL	1 mL	2 mL	5 mL	10 mL

Figure 6: A table showing aspirated volume for a range of reservoir pressures and volumes.

As is evident, the larger the reservoir volume, the lower the aspiration pressure required to aspirate a given liquid volume. On one hand, selecting as large a reservoir as possible (given other constraints e.g. acceptable system size) is attractive, as it reduces the pump drive power required to maintain the aspiration pressure. On the other hand, dosing precision depends on how precisely the aspiration pressure is measured and controlled – clearly, a lower aspiration pressure will require a higher-resolution pressure sensor.

In addition, to ensure a good signal-to-noise ratio (SNR) on the pressure measurement, the maximum aspiration pressure should be a good fraction of the full-scale range of the pressure sensor.

3.6.2. Orifice Selection

When selecting an orifice, there is a trade-off to consider between the desired dispense speed, and the effect of self-heating due to the pump. On one hand, a larger orifice enables the pressure in the reservoir to dissipate more quickly, supporting faster dispense speeds. On the other hand, with a larger orifice, the pump will need to work harder to maintain pressure in the reservoir between the aspirate and dispense steps. As a result, the pump will generate more heat, which in turn will heat the gas flowing through the pump, reservoir, and orifice. This will contribute to a temperature difference between the gas in the reservoir (T_r) and the gas in the pipette tip (T_s), which will impact pipetting precision (without further temperature compensation).

Dispense speed can be calculated as follows:

$$T_D = 15\ L\sqrt{V_{asp}V_r}$$

Where T_D is the dispense time in milliseconds, L is the feedback orifice restriction in Lohms, V_r is the reservoir volume in litres, and V_{asp} is the fluid aspiration volume also in litres.

Using this equation, Figure 7 shows the maximum reservoir volume that should be used to achieve a desired dispense speed for a range of common orifice sizes, for an aspiration volume (V_{asp}) of 100µL.

Maximum Reservoir Volume for Aspirating 100µL									
Dispense Time	Feedback Orifice Restriction - LOhms								
	4k	8k	12k	15k	20k	25k	30k	40k	45k
20ms	1.1mL	277µL	123µL	79µL	44µL	28µL	20µL	11µL	8.8µL
50ms	6.9mL	1.7mL	772µL	494µL	278µL	178µL	123µL	69µL	55µL
100ms	28mL	6.9mL	3.1mL	2.0mL	1.1mL	711µL	494µL	278µL	219µL
200ms	111mL	28mL	12mL	7.9mL	4.4mL	2.8mL	2.0mL	1.1mL	878µL
500ms	694mL	174mL	77mL	49mL	28mL	18mL	12mL	6.9mL	5.5mL
1s	2.8L	694mL	308mL	198mL	111mL	71mL	49mL	28mL	22mL
2s	11L	2.8L	1.2L	790mL	444mL	284mL	198mL	111mL	88mL

Figure 7: A table showing the maximum reservoir size for dispensing 100µL with a desired dispense time. The maximum volume is also inversely proportional to the aspirated volume so other volumes should be easily calculated from this table.

Note that the orifice value itself does not directly affect V_{asp}, only the power needed to achieve the desired ΔP_r. As a result, that the exact value of the orifice is not critically important: this means that it is possible to use a lower-precision orifice if desired.

3.6.3. Pump Selection

The Lee Company recommends that VCM modules are made with parallel-configuration (PDC) pumps (this position is explained in the appendix). Pump models UBLB5400200A and UXPB5400200A are therefore ideal. Figure 8 shows the performance supported by UBLB5400200A for a range of orifice sizes and drive powers. We recommend operating the pump at/below 250 mW drive power, to minimise the effect of pump self-heating (as described in 3.3.1).

Pressure (mBar) across orifice for UBLB5400200A									
Drive Power	Orifice - LOhms								
	4k	8k	12k	15k	20k	25k	30k	40k	45k
20mW	4	6	7	8	8	9	9	9	9
50mW	8	13	15	16	16	17	17	18	18
100mW	15	22	25	26	27	28	29	30	30
250mW	32	44	49	51	53	55	56	57	57
500mW	57	75	82	85	88	90	91	93	94
1W	99	126	136	141	145	148	150	152	153

Figure 8: Using the specification performance of a UBLB5400200A pump (160mBar stall pressure and 1.65slpm free flow) the pressures across different orifice sizes were calculated for a range of pump drive powers. The colour of the row represents how likely the drive power is to cause problems due to self-heating of the pump (green equates to little self-heating; red equates to larger levels of self-heating).

Similar tables for other PDC pump models are presented in **Appendix B**.

3.7. Example systems

In this section, we work through the selection of components for three example systems with differing requirements and configurations.

	Design A	**Design B**	**Design C**
Required Performance	Aspirate 50-200µL And dispense in 500ms	Aspirate 500µL Dispense in 200ms	Aspirate 10µL with highest possible accuracy
Resulting Design	UBLB5400200A V_r = 10mL 20kLohm Orifice	UBLB5400200A V_r = 20mL 4kLohm Orifice	UBLB5400200A V_r = 0.2mL 45kLohm Orifice

Figure 9: Three example system requirements and configurations, considered in further detail below.

3.7.1. Design A

In our first example, the required performance is to aspirate fluid volumes of 50µL to 200µL, and to dispense the volume in half a second (500ms).

In this case, we start by considering the lower target aspiration volume of 50µL. Using Figure 6, we select a reservoir volume of 10mL to give a low-but-measurable aspiration pressure of 5 mBar. Then, with the upper end of the target aspiration volume range of 200µL, we use the equation in §**Error! Reference source not found.** to calculate the orifice size required to give the correct dispense speed:

$$T_D = 15\, L\sqrt{V_{asp}V_r}$$

Therefore: $$L = \frac{T_D}{15\sqrt{V_{asp}V_r}} = \frac{500}{15\sqrt{0.0002*0.01}} = 23570\ Lohms$$

We choose the largest standard orifice Lohm rating below the calculated value. In this case, a 20kLohm orifice will be suitable. Consulting Figure 6 once again, we note that the pressure required to aspirate 200µL with a 10mL reservoir is 20mBar. Figure 8 shows that the pump drive power required to generate 20mBar across a 20kLohm orifice with UBLB5400200A is less than 100mW – this is lower than 250mW, which means that any effects due to self-heating should be small.

Finally, we should select a pressure sensor with an appropriate full-scale range: 30 to 50 mBar would be appropriate, given the maximum aspiration pressure of 20 mBar.

3.7.2. Design B

In the second example, the requirement is to aspirate 500µL and then to dispense this in 200ms.

In this case, we first identify the largest reservoir that will support the desired dispense speed. Using the equation in §**Error! Reference source not found.** and selecting a small orifice size of 4kLohm, we calculate that the reservoir size:

$$T_D = 15\, L\sqrt{V_{asp}V_r}$$

Therefore: $$V_r = \frac{{T_D}^2}{225\, L^2\, V_{asp}} = \frac{200^2}{225 \times 4000^2 \times 0.0005} = 0.022\ L = 22\ mL$$

Therefore, the reservoir should be ≤ 22mL. We therefore choose a 20mL reservoir. Using the equation in 3.3, we can calculate the pressure required to aspirate 500µL:

$$V_{asp} = \frac{V_r \Delta P_r}{P_s}$$

Therefore: $\Delta P_r = \frac{V_{asp} P_s}{V_r} = \frac{0.005 \times 1}{0.02} = 0.025\ Bar = 25\ mBar$

Finally, consulting Figure 8 we can see that the pump drive power required to generate 25 mbar across a 4kLohm orifice, using a UBLB5400200A pump, is less than 250mW; this means that any effects due to self-heating should be small.

This system uses a large reservoir to combat self-heating from the pump, however, note that large reservoirs will also be more susceptible to changes in ambient temperature.

3.7.3. Design C

In the third example, the requirement is to aspirate 10µL with the highest accuracy possible.

In this case, it is a good idea to operate with a high aspiration pressure so that the impact of pressure sensor imprecision is minimised. At the same time, we should be mindful of the drive power required to reach the aspiration pressure (given the orifice selected): we should still aim for this to be lower than 250mW to avoid significant self-heating.

Consulting Figure 8, we can see that an aspiration pressures of ≤57mBar with a 45kLohm orifice will require drive power ≤250mW for a UBLB5400200A, so this sets our upper pressure limit. We choose 50mBar to provide a little margin. Using the equation in 3.3, we can calculate the reservoir volume required to aspirate 10µLwith 50mBar:

$$V_{asp} = \frac{V_r \Delta P_r}{P_s}$$

Therefore: $V_r = \frac{V_{asp} P_s}{P_r} = \frac{0.00001 \times 1}{0.05} = 0.0002\ L = 200\ \mu L$

Therefore the reservoir volume should be 200μL. We note that this is a small reservoir and will require manufacturing and system assembly precision; however, given pipette tips of this volume are available, we conclude that it should be possible to manufacture a system reservoir with similar (and therefore adequate) precision. We calculate that this setup will provide a dispense time of less than 50ms.

Note that while this configuration is suitable for aspirating 10μL (and volumes lower than this), it is likely unable to aspirate volumes that are significantly larger than 10 μL without suffering from self-heating effects.

3.8.Pitfalls to avoid

There are a number of possible pitfalls to avoid when designing and operating the VCM as follows:

1. Self-heating that occurs when running the pump at higher drive levels, which can raise the temperature of the gas in the reservoir and cause drift in the volume of fluid aspirated.
2. Leaks from the reservoir to the outside world, which can result in a continuous increase in the aspirated volume in order to keep the reservoir pressure at the required setpoint.
3. Leaks from the system side (e.g. at the interface between the pipette tip and the module it connects to) causing the aspirated volume to drop whilst being held in the pipette tip.
4. Compliance of the reservoir and/or connecting pathways, resulting in expansion on pressurisation.
5. Environmental temperature changes

3.8.1. Self-heating

As the pump runs, a portion of the drive power goes directly to heat. In addition, as air returns through the feedback orifice more energy is lost to heat. As a result, the temperature of the air circulating through the reservoir, pump and orifice will increase.

As its temperature increases, the gas expands. Given that the reservoir volume is fixed, the expanding gas causes the pressure in the reservoir to rise. Given that pump is driven to control the reservoir to set pressure, the pump drive level is reduced, allowing gas to flow from reservoir side to the system side. This has the effect of gradually reducing the aspirated volume over time as the circulating gas heats up, as shown in Figure 10.

This can be countered by using a larger reservoir, as for a given aspiration volume, the aspiration pressure required will be lower; this means that the pump can be driven more gently, reducing the amount of heat that the pump generates. In addition, the thermal mass of the circulating air will be larger, slowing the temperature rise.

Whilst a larger reservoir can counter self-heating, it does make the VCM more susceptible to ambient temperature swings (or heating from other active components that may be nearby) whilst pressure is being held in the reservoir. For the ambient thermal effects to be small it is needed that:

$$\frac{\Delta T_{therm}}{T_0} < \frac{\Delta P_{res}}{P_0}$$

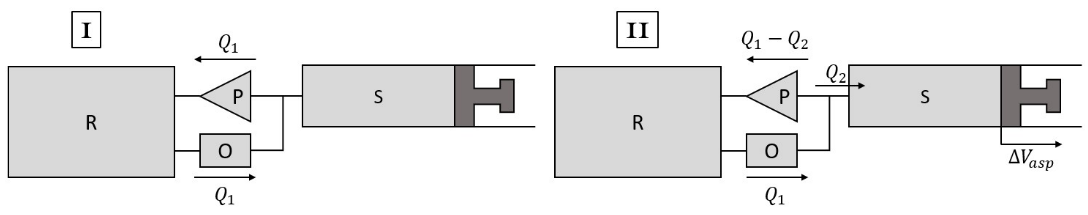


Figure 10: Diagram I shows the situation without heating, where the flow through the pump and the feedback orifice balances, such that there is no flow in or out of the system.

In Diagram II, the effects of heating are shown: as the air in the reservoir heats up, the pressure of the air within it increases. As a result, the pump doesn't have to work as hard to maintain the reservoir pressure setpoint, meaning that less gas is driven though the pump into the reservoir. The flow through the orifice remains the same, as this is driven by the reservoir pressure. As a result, there is a net flow out of the reservoir and into the system.

3.8.2. Leaks

If the system is perfectly sealed, then when the reservoir pressure is held constant, the flowrate through the pump will be equal to the flow leaving the reservoir through the feedback orifice. This means that the amount of gas in the system will remain constant, as in diagram 1 in Figure 11.

If there is a leak in the reservoir (or between the reservoir and pump, or reservoir and orifice, etc) then the flowrate of the pump will no longer be equal to the flowrate through the feedback orifice. If the reservoir pressure is kept constant, then the flow through the pump will increase to match the leak rate. This causes the fluid aspiration volume to increase gradually as in diagram 2 of Figure 11.

A leak on the system side would cause the volume aspirated to drop, due to extra gas entering the system, shown in diagram 3 of Figure 11. However, this kind of leak is likely to present less of an issue, as the pressure in the system will (in most cases) be much closer to ambient pressure, causing the leak rate to be smaller.

Preventing leaks in the VCM critically important to ensure good, precise performance. This is especially true where the aspiration volume is small: for example, when aspirating 10μL, it only takes 6 seconds for a 1μL per minute leak to cause a 1% change to the volume aspirated. Leaks this small can be hard to measure—and indeed prevent.

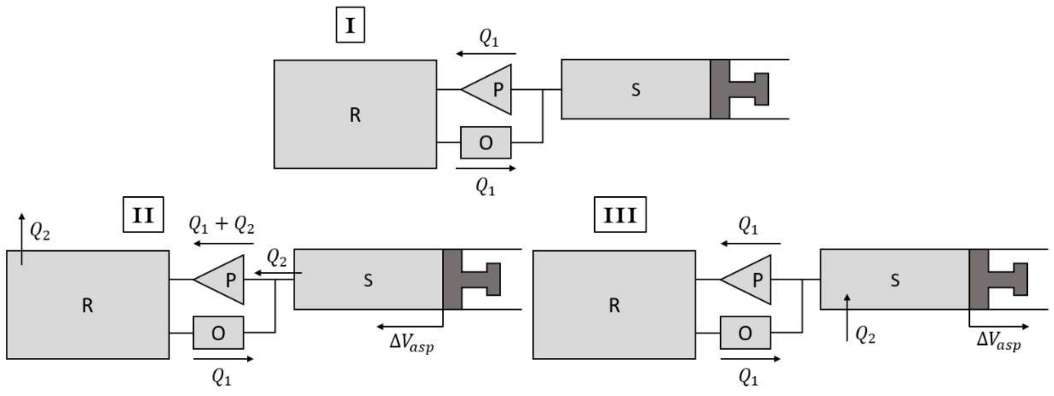


Figure 11: Diagram I Shows the situation with no leaks, where the flow through the orifice and the pump is equal.

Diagram II shows the case of a leak out of the reservoir. Diagram III shows a leak into the system side. If the fluid being aspirated against gravity, there will be a small negative pressure in the system side which could drive a leak in the opposite direction to diagram II.

3.8.3. Compliance

If the reservoir (or linking fluidic pathway) is made of a compliant material, then as the reservoir pressure (P_r) increases, the volume of the reservoir (and/or linking pathway) will also increase. As a result, the volume aspirated is no longer linearly proportional to the pressure in the system, given the volume of the system is now a function of the pressure.

This is easily prevented by using stiff materials and constructions that do not expand readily with pressure.

4. DATA

Two main embodiments of this system have been built and tested, as per the diagrams in Figure 12:

1. In the first embodiment, the mass of water aspirated by a pipette tip was measured using a high precision mass balance. Two balances were used: one with 0.1 mg sensitivity, and one with 1 mg)
2. In the second embodiment, the total amount of fluid moved is measured with a high-precision liquid flow sensor.

The first embodiment uses a pipette tip, mimicking the real-world use case we anticipate for the VCM. However, with this approach we found inconsistencies in the interaction between the fluid and the pipette tip—such as the formation of droplets, liquid remaining on the interior sidewall of the tip, and other effects related to surface tension. We believe these inconsistences cause variation between pipetting cycles that appear to limit repeatability / precision.

Therefore, the second embodiment is designed to show the potential repeatability of the system by removing complications due to fluid-tip interaction.

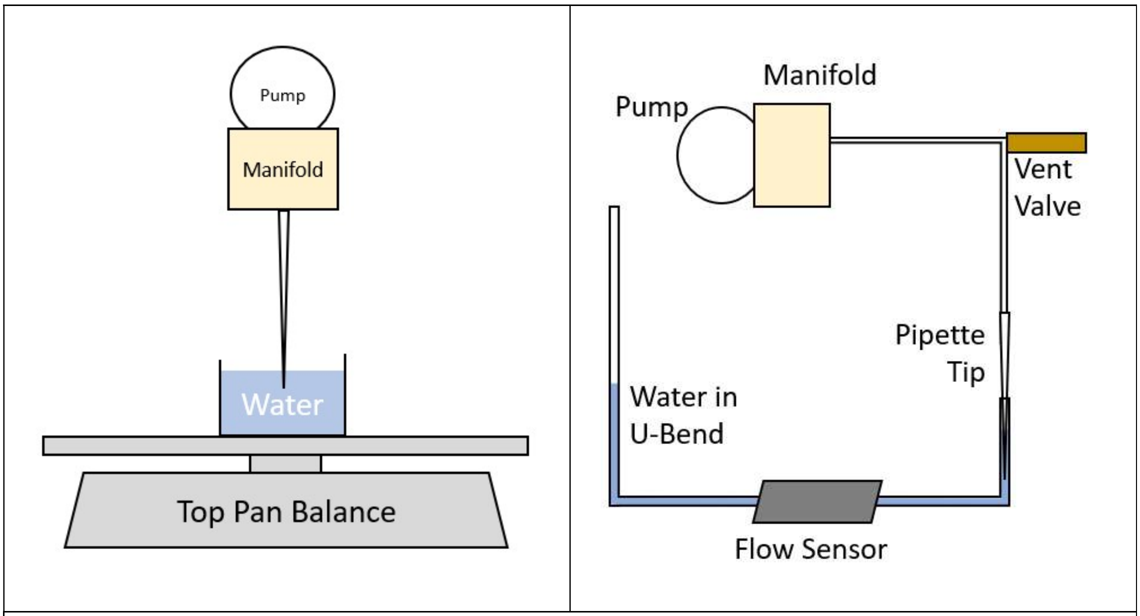


Figure 12: Schematics for the two different test systems. The left-hand schematic is the first embodiment, where the mass of the fluid aspirated is measured with a mass balance. The right-hand schematic is the second embodiment, designed to reduce variability in the data by integrating the output of a high-precision flow sensor to measure the total amount of fluid moved around a U-bend.

4.1.Data with Pipette tips

In this test a VCM with a reservoir volume of ~1.5mL and an *ART Molecular BioProducts 200L* tip was used. The amount of liquid aspirated was measured by lowering the pipette tip into a bath of water located on the mass balance. The water was then aspirated into the tip and the mass difference measured.

In the data presented in Figure 13, four different aspiration pressure setpoints were measured. There was a good linear correlation between ΔP_r and V_{asp}. Figure 14 shows the spread in the data more clearly, with the range being approximately ±2 to 3mg from the mean. This gives a lower limit on the aspirated volume that can be achieved with a Coefficient of Variation (CV) of less than 1%, of 100-200µL.

However, during this test we noticed inconsistencies in the interaction between the fluid and the pipette tip—such as fluid pinning in the tip, droplets remaining on the interior sidewall of the tip after dispensing, and other effects related to surface tension. We believe these inconsistences contributed to a significant fraction of the variation that limited repeatability between pipetting cycles.

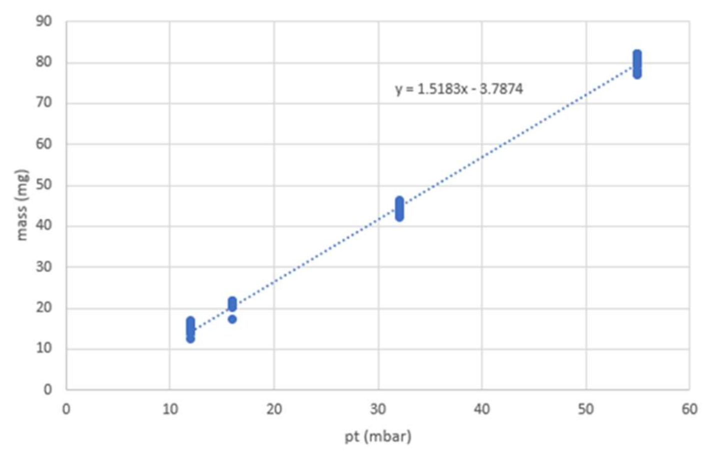


Figure 13: A graph showing the mass aspirated versus aspiration pressure. The graph shows a good linear correlation between the pressure and the aspirated mass. There is a visible spread in the datapoints for each pressure setpoint.

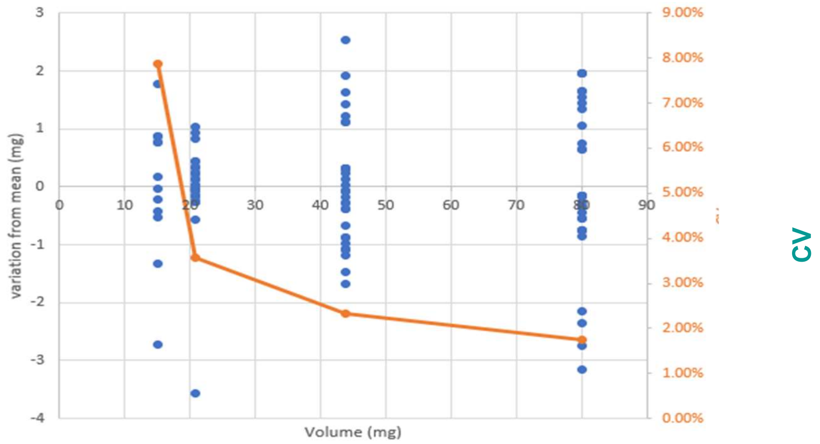


Figure 14: A graph showing the spread of the datapoints from the mean in blue and the CV for the given setpoint in orange.

4.2. Data with a Liquid Flow Sensor

To determine the impact that inconsistent fluid-tip interaction has on repeatability, a second VCM embodiment was designed to remove fluid-tip interaction altogether. In this embodiment, the tip is not inserted into / removed from the fluid during each pipetting cycle. Instead, the tip is in permanent contact with a body of fluid in a 'U'-bend shaped tube, as shown in the right-hand schematic in Figure 12. The flow rate of fluid "aspirated/dispensed" was measured with a high-precision flow sensor, and then the flow sensor output was integrated to give the total volume moved through the U-bend.

In this embodiment, there was an excellent linear correlation between the reservoir pressure and the volume "aspirated/dispensed" as can be seen in Figure 15. Note that the gradient of this curve is different to that in Figure 13, as the reservoir volume was different.

The standard deviation attained by this method were significantly improved, leading to better CV values, as can be seen in Figure 16. The measured CVs were lower than 1% across the full range of aspiration volumes, from ~15μL to ~400 μL.

The results for this measurement method were much more repeatable. This provides confidence that the VCM is capable of high-precision operation, and that real-world precision may be limited by fluid-tip interactions, and by the measurement precision of the mass balance used in the first embodiment.

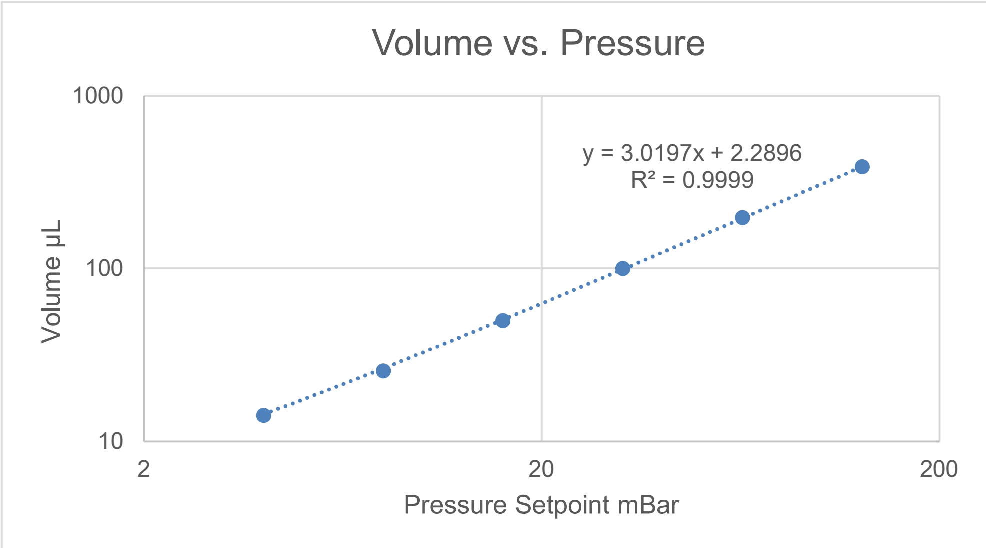


Figure 15: A log plot showing the aspirated volume vs. the reservoir pressure giving an excellent linear correlation, for data taken with a liquid flow meter.

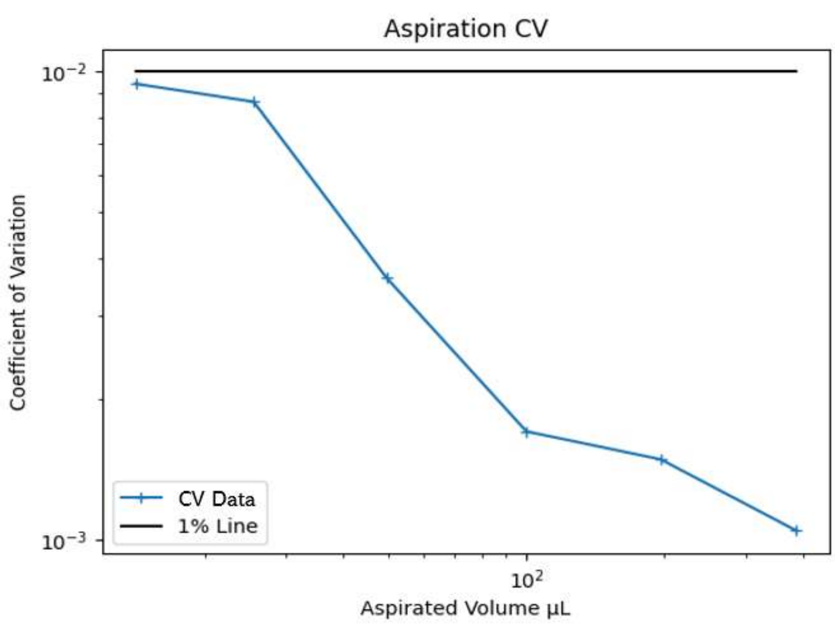


Figure 16: A log plot showing the CV against aspirated volume for data taken with a liquid flow meter.

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4.3.Thermal Stability

Using the first experimental setup, the effect of temperature on the VCM was tested. In theory, if the reservoir and system volumes are at the same temperature, there should not be any thermal effect on the pressure needed to aspirate a given volume.

The results of this test are presented in Figure 17. The test was conducted at two temperatures: room temperature, and 40°C. Despite the ~7% increase in absolute temperature, the gradient of the curve (for reservoir pressure versus aspirated volume) only increased by ~0.7%. This change is within the measurement noise of the scales and is therefore considered insignificant. This provides evidence that ambient temperature changes do not affect the VCM, providing that the reservoir and system volumes are at the same temperature.

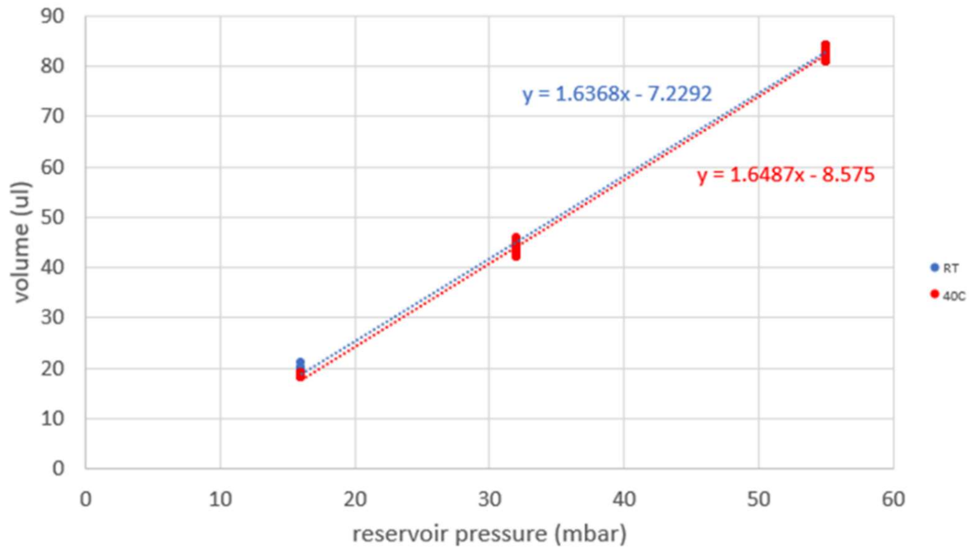


Figure 17 shows the effect of temperature changes on the VCM. As long as the temperature of the reservoir and system volumes is consistent, then no significant thermal effects are observed. This data does show a slight difference between the two temperatures; however this difference is within the error of the scales and is significantly smaller that the relative change in absolute temperature.

The standard deviation of the mass aspirated was also unchanged by raising the temperature.

4.3.1. Thermal Expansion

Whilst the fundamental operation of the VCM has been shown to be independent of the ambient temperature, if the materials selected for the VCM exhibit significant thermal expansion, then changes in temperature may still have a significant effect on pipetting repeatability. Therefore, care should be taken when selecting materials, considering the range of temperatures that the system is designed to operate in.

In our thermal test the reservoir was a 3D printed in SLA part. The material used expands by approximately 0.5% when heated from room temperature to 50 C. If instead the reservoir was made from steel, the amount it would have expanded by would have roughly been 0.05%, i.e. 10x less, which would result in improved pipetting repeatability across temperature.

5. USEFUL DESIGN ADDITIONS

This document has covered a minimal viable VCM design. There are a several additions that can be made to the system to enable useful functionality.

5.1.Valving

5.1.1. Side Vent Valve

Step 1 of the VCM process in §3.5 is a pre-charge step, intended to enable full and complete dispensing during Step 5. Whilst this technique works well, it does require the pump to run harder to maintain a higher aspiration pressure whilst holding fluid in the pipette tip. This will increase the amount of heat generated by the pump. For many applications, this temperature rise will be negligible. For more demanding applications that require higher pump drive levels, it may reduce pipetting accuracy and repeatability as discussed in §3.8.1.

To mitigate this issue, a side vent valve can be used as shown in Figure 18. This enables the VCM to draw air from the atmosphere rather than from the pipette tip, making it possible to increase the pressure in the reservoir without aspirating more air from the system (and therefore without aspirating more liquid). As a result, the additional ‘charging’ step required for full and complete dispensing can be carried out *after* aspirating the fluid, and immediately prior to dispensing. This minimises the length of time that the pump runs at higher power, reducing heating and maximising pipetting performance.

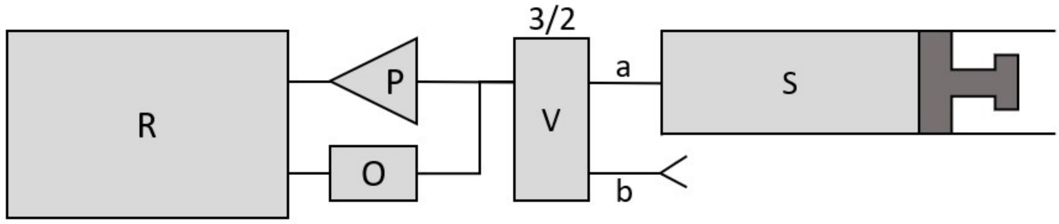


Figure 18: Showing a 3-2 valve, V, between the VCM and the system, allowing more air to be aspirated from atmosphere (b) after the desired amount of air has been aspirated from the system (a). This allows for blow-off, which can enable a more effective and complete dispense.

5.1.2. Feedback Valve

If the application requires the aspirated volume to be held for large periods of time, it may not be possible to limit the heating effects of the pump with the methods already described. This will lead instability in the aspirated volume.

One way to counter this is with a valve, either in parallel or series with the feedback orifice. If the valve is in series with the feedback orifice, then the valve may be closed and the pump turned off once the required pressure is reached in the reservoir. Then, when it comes to dispensing the volume, the valve can be opened and the gas allowed to escape the reservoir. This setup has the disadvantage that any backflow through the pump will need to be countered by turning the pump back on, so as to top up the reservoir pressure. Therefore the VCM should continuously monitor the reservoir pressure during the hold stage.

If instead the valve is placed in parallel with the feedback orifice, then a much more restrictive orifice can be selected than dispense speed would otherwise require (e.g. per Figure 7). This allows the pump to be operated at a much lower drive power than with a higher-flow orifice, thereby reducing heating. When dispensing, the valve is opened, allowing the reservoir pressure to discharge quickly, supporting rapid dispense speeds.

5.1.3. Multi-Volume Valve

A valve can be placed between the main reservoir and second reservoir (as shown in Figure 19), which allows for different fluid volumes to be aspirated while still running the pump at optimal pressure for self-heating and pressure sensor accuracy.

A second feedback orifice could also be fitted to the second reservoir to allow for a greater dispense speed if so required.

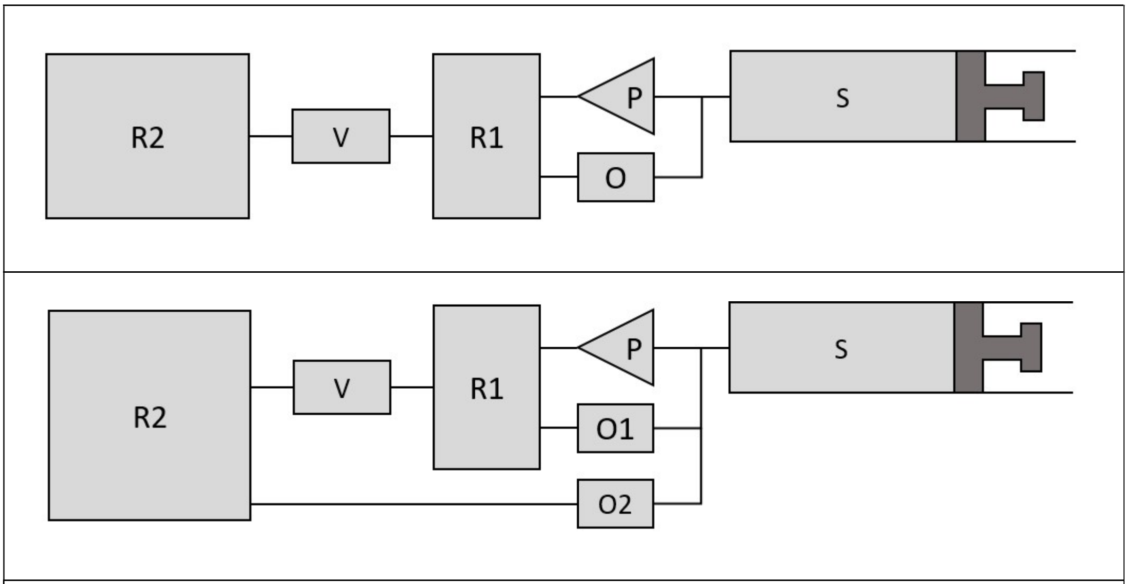


Figure 19: The top diagram shows a setup with two reservoirs and valve, V, between them. This allows the system to operate with two different reservoir volumes. The bottom diagram shows a similar system but with a second feedback orifice to allow for fast dispense time with a larger reservoir.

5.2. Temperature measurement

If it is expected that the ambient temperature of the system and the reservoir will be different (e.g. because the fluid is cooler or hotter than the ambient temperature, for example) it may be prudent to measure the actual gas temperatures of the VCM and system—for example with a pair of thermocouples. Any temperature difference between the VCM and system will have an impact on the volume aspirated per unit pressure. By measuring these temperatures, it is possible to calculate and apply appropriate temperature compensation.

It is also possible to use thermocouples to measure any self-heating of the module. Applying temperature compensation for this measurement may be more complicated, however, as it requires knowledge of the full internal volume of the system (e.g. including the internal volume of the pump and connecting fluidic pathway). In addition, the air in the pump chambers will be at a significantly higher temperature than the reservoir and so will account for a large portion of the thermal expansion for systems with a small reservoir volume. Nevertheless, in principle it is still possible to apply temperature compensation for self-heating where this is necessary.

Measuring the temperature of the air in the reservoir requires that it is well mixed. A good way to ensure this is to have the gas travel though most of the reservoir on its journey from the pump to the feedback orifice (Figure 20).

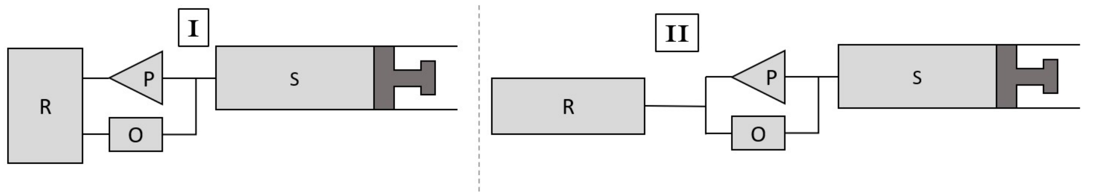


Figure 20: Diagram I shows a system in which the air in the reservoir will be well mixed, as the inlet to, and outlet from, the reservoir are spaced apart, causing the air to take a longer path through the reservoir, which promotes mixing. Diagram II shows a situation in which the air will not be well mixed, as the circulating air can bypass the reservoir altogether it on its way to the feedback orifice.

6. USEFUL EQUATIONS

6.1. Aspirating Against Gravity

If the liquid being aspirated is being lifted against gravity, then aspiration pressure will need to be higher to be able to hold the liquid against gravity. Unless the pipette tip is particularly long, the effects should be small. Nevertheless, it is easy to calculate and therefore compensate for:

$$\mathrm{V_r}\Delta P_r = P_0 V_{asp} + \frac{\rho g V_{asp}}{A}\left[V_s - V_{asp}\right]$$

Where A is the cross-sectional area of the pipette tip and ρ the density of the fluid. The terms in red are the addition terms relating to the interaction with gravity.

For the gravity effects to be large, these extra terms need to be significant when compared to the standard terms. Therefore, to be able to ignore gravity effects it is required that:

$$\frac{\rho g}{AP_0}\left[V_s - V_{asp}\right] \ll 1$$

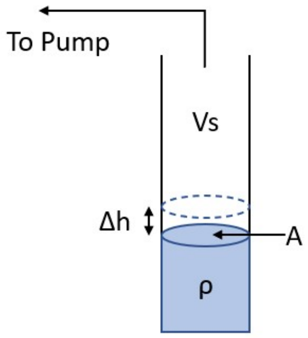
An example system acting on water and consisting of a 10cm long uniform cross section pipette tip at atmospheric pressure would give:

$$\frac{\rho g}{AP_0}\left[V_s - V_{asp}\right] \approx \frac{\rho g V_s}{AP_0} = \frac{\rho g H}{P_0} \approx \frac{10^3 \cdot 10 \cdot 10^{-1}}{10^5} = 10^{-2} = 1\%$$

Where H is the height of the tube, and it is assumed that $V_s \gg V_{asp}$. In this system the effect is small, yet not insignificant for a high precision pipetting system.

Figure 21: Showing the simplified system for gravity aspiration and the key factors labelled.

$$V_{asp} = A\Delta h$$



6.2. Dispense / Deflation through an Orifice

For air at room temperature, if the units are:

- slpm for flowrate.
  - Litres for volume
  - Minutes for time
- Bar for pressure.

Then the flowrate through an orifice can be calculated[1] as:

$$Q = \frac{8000}{L}\sqrt{P_{atm}\Delta P_r} = \frac{8000}{L}\sqrt{\Delta P_r}\ (\text{if } P_{atm} = 1Bar)$$

This then gives a deflation time, for a given reservoir of volume V_r and initial pressure of ΔP_r, of

$$T_D = \frac{LV_r\sqrt{\Delta P_r}}{4000}$$

This can then be rewritten in terms of V_{asp} rather than ΔP_r, and with time units in milliseconds rather than minutes:

$$T_D = 15\ L\sqrt{V_{asp}V_r}$$

Leading to the equation given in a previous section.

Therefore for a 10cc reservoir, a 4K orifice and an aspirated volume of 100μL.

- L = 4000
- V_r = 0.01 Litre
- V_{asp} = 0.0001 Litre

gives a T_D of 60ms.

6.3. Orifice Pressure/Flowrate vs. Pump Power

The pressure that a pump will be able to generate against an orifice is calculated by intersection of the orifice flow curve (shown in Figure 4). The orifice line is given in the previous section as,

$$Q = \frac{8000}{L}\sqrt{\Delta P_r}$$

And the pump load line at a given power, which if assumed to be a straight line in pressure vs. flowrate, is given by,

---

[1] See How to Calculate Flow Resistance for Gases | The Lee Co, using the subsonic equation.

$$P = P_{stall} - \frac{P_{stall}}{Q_{free}} Q$$

Where P is set to $= \Delta P_r$, P_{stall} is the stall (maximum) pressure of the pump and Q_{free} is the flowrate with no back pressure. This can be solved to give the pressure that would be in the reservoir if the pump was run against a given orifice.

$$\Delta P_r = 2\left(\frac{8000P_s}{2Q_f L}\right)^2 - 2\left(\frac{8000P_s}{2Q_f L}\right)\sqrt{\left(\frac{8000P_s}{2Q_f L}\right)^2 + P_s}$$

P_{stall} and Q_{free} can be approximated at different powers (Π) using,

$$P_\Pi = P_{stall} e^{0.7\Pi} \; and \; Q_\Pi = Q_{free} e^{0.55}$$

6.4.Thermal Effects

If it is assumed that the gas in the reservoir is the only gas being heated then the fraction of the initial aspirated gas that subsequently flows back to the pipette tip is:

$$\frac{n_{therm}}{n_{asp}} = \frac{P_0 + \Delta P_r}{\Delta P_r} \cdot \frac{\Delta T_r}{T_0 + \Delta T_r}$$

This can be approximated as:

$$\frac{n_{therm}}{n_{asp}} = \frac{P_0 + \Delta P_r}{P_0} \cdot \frac{\Delta T_r}{T_0 + \Delta T_r} = \frac{\Delta T_r}{T_0} \cdot \frac{1 + \frac{P_0}{\Delta P_r}}{1 + \frac{\Delta T_r}{T_0}} \approx \frac{\Delta T_r}{T_0} \cdot \left(1 + \frac{P_0}{\Delta P_r}\right) = \frac{\Delta T_r}{T_0} \cdot \left(1 + \frac{V_r}{V_{asp}}\right)$$

As the increase in temperature for a given amount of heat will be inversely proportional to the volume of gas ($\Delta T_r = k/V_r$) that is heated, this means that the fraction de-aspirated due to heating is given by:

$$\frac{n_{therm}}{n_{asp}} = \frac{k}{V_r T_0} \cdot \left(1 + \frac{V_r}{V_{asp}}\right) = \frac{k}{V_r T_0} + \frac{k}{V_{asp} T_0}$$

The amount of heating is also proportional to the power of the pump that is roughly proportional to the square of the pressure, meaning that $k \propto (\Delta P_r)^2 \propto V_r^{-2}$ giving:

$$\frac{n_{therm}}{n_{asp}} \propto \frac{1}{V_r^3} + \frac{1}{V_{asp} V_r^2} = \frac{1 + V_{asp}/V_r}{V_{asp} V_r^2} \approx \frac{1}{V_{asp} V_r^2} \quad (if\ V_r > V_{asp})$$

This means that heating effects will get smaller with a larger reservoir.

6.5.Accounting for Compliance

If the material used is stiff enough that the expansion can be approximated as linear, then the volume aspirated is given by:

$$V_{asp} = \frac{T_s}{T_r P_s} \cdot V_r\left(\Delta P_r + \alpha_{\Delta P_r} P_r\right)$$

Where $\alpha_{\Delta P_r}$ is the fractional expansion of the reservoir when pressurised to the target pressure. In this case V_r is specifically the initial reservoir volume, and P_r is the absolute pressure in the reservoir once it is filled.

It may be possible to calibrate a system if very high precision is needed, however it would likely be easier to use a material stiff enough to not expand. If aspirating to 20mBar, then for over-aspiration to reach 1% due to expansion, the fractional expansion $\alpha_{\Delta P_r}$ would need to exceed 0.0196, which would be a large expansion for such a low pressure for any reasonable material.

7. APPENDIX

7.1.PDC VS SDC

As this is a pressure-limited rather than flowrate-limited system, it might appear tempting to try and use a higher pressure pump (e.g. an SDC or even a HP Series pump) instead of a PDC. However, this is not a good idea. This is because Disc Pumps have internal volume: in the case of a PDC pump this is a small volume before the valve, and adds to the system-side volume. In an SDC pump, this volume is between

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the valves (as shown in Figure 22) and, during operation, is at a undefined and hard-to-measure pressure. This reduces control precision and places a minimum value on the effective reservoir volume.

This is also true for HP Series and UltraSlim Series pumps.

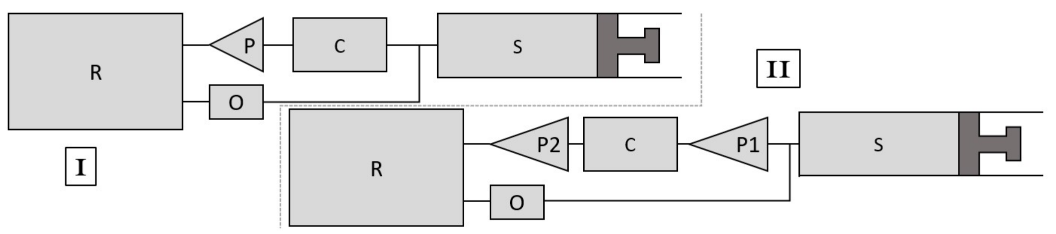

Figure 22: Diagram I shows a schematic of a VCM using a PDC pump. The volume inside the pump connected directly to the system so causes minimal complication. Diagram II shows the situation with an SDC pump; the volume C is between two halves of the pump so and is at an unknown pressure between P_S and P_R, and also gives a lower bound on the effective reservoir volume.

7.2.PERFORMANCE TABLES FOR ALL PDC PUMP

Values given are approximate values, calculated from pump minimum product specification and assuming a straight load line.

UBLB5400000A: BL series, max power 1W

Stall Pressure (mBar) of an UBLB5400000A									
Drive Power	Orifice - LOhms								
	4k	8k	12k	15k	20k	25k	30k	40k	45k
20mW	2	4	6	7	8	8	9	9	9
50mW	5	10	12	14	15	16	17	18	18
100mW	9	17	22	24	26	27	28	30	30
250mW	22	37	44	48	51	54	55	57	58
500mW	40	64	75	80	86	89	92	95	96
1W	73	111	127	135	143	148	151	156	157

UBLB5400200A: BL series, max power 1W

Stall Pressure (mBar) of an UBLB5400200A									
Drive Power	Orifice - LOhms								
	4k	8k	12k	15k	20k	25k	30k	40k	45k
20mW	4	6	7	8	8	9	9	9	9
50mW	8	13	15	16	16	17	17	18	18
100mW	15	22	25	26	27	28	29	30	30
250mW	32	44	49	51	53	55	56	57	57
500mW	57	75	82	85	88	90	91	93	94
1W	99	126	136	141	145	148	150	152	153

UXPB5400000A: XP series. Max power 1.4W intermittent, but not recommended for a VCM due to self-heating.

Stall Pressure (mBar) of an UXPB5400000A									
Drive Power	Orifice - LOhms								
	4k	8k	12k	15k	20k	25k	30k	40k	45k
20mW	3	5	7	8	9	9	10	10	11
50mW	6	12	14	16	18	19	19	20	21
100mW	12	20	25	27	30	31	32	33	34
250mW	26	43	51	55	58	61	63	65	66
500mW	48	74	86	92	98	101	104	107	108
1W	88	128	146	154	162	167	171	176	177
1.4W	116	166	188	197	207	214	218	223	225

UXPB5400200A: XP series. Max power 1.4W intermittent, but not recommended for a VCM due to self-heating.

Stall Pressure (mBar) of an UXPB5400200A

Drive Power	Orifice - LOhms								
	4k	8k	12k	15k	20k	25k	30k	40k	45k
20mW	4	6	7	7	8	8	8	9	9
50mW	8	12	14	15	16	16	16	17	17
100mW	15	21	24	25	26	27	27	28	28
250mW	31	42	47	48	50	52	52	54	54
500mW	55	71	78	80	83	85	86	88	88
1W	95	119	129	133	137	139	141	143	144
1.4W	124	153	165	169	174	177	179	182	183

The 1.4W intermittent powers for XP series could be used to generate large blowoff pressures in conjunction with a valve.

8. SUPPORT

The Lee Company website provides advice on:

- Getting Started
- Applications
- Development Process
- Downloads (including datasheets, application notes, case studies and 3D models)

The Lee Company is happy to discuss next steps beyond prototyping, including system design. If you would like to discuss this with us, or for any other additional support, please contact your Lee Sales Engineer.

9. REVISION HISTORY

Date	Version	Change
June 2023	06/23	Rebranded document
13 April 2022	220413	First release of detailed application note.