Optimization of Liquid Handling Parameters of Pipetting Robots to Transfer Viscous Liquids, a "Sticky Situation"

Electronic Supporting information

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CONTENTS

S1 MATERIALS AND EQUIPMENT······1
S1.1 Viscosity standards······1
Table S1······1
S1.2 In-house assembled liquid transfer platform ······2
Figure S1······2
S1.3 In-house built automated mass balance······2
Figure S2······2
S2. EXPERIMENTAL······1
S2.1 Workflow diagrams······1
Figure S3······1
S2.2 Flow rate control for rLine1000 pipette······2
Figure S4······2
S2.3 Automated flow rate calculation······4
Figure S5······2

S3 RESULTS······5
Figure S6······5
Figure S7······5

S1 MATERIALS AND EQUIPMENT

S1.1 Viscosity standards

Standard name	Viscosity @25°C	Density @25°C	Viscosity @20°C	Density @20°C
Viscosity standard 204	204.8	0.8639	285.6	0.8664
Viscosity standard 505	505.4	0.8683	727.1	0.8713
Viscosity standard 817	817.4	0.8466	1136	0.8476
Viscosity standard 1275	1275	0.8736	1899	0.8765

Table S1. Physical properties (viscosity and density) of viscous liquids standards used in this study.

S1.2 In-house assembled liquid transfer platform

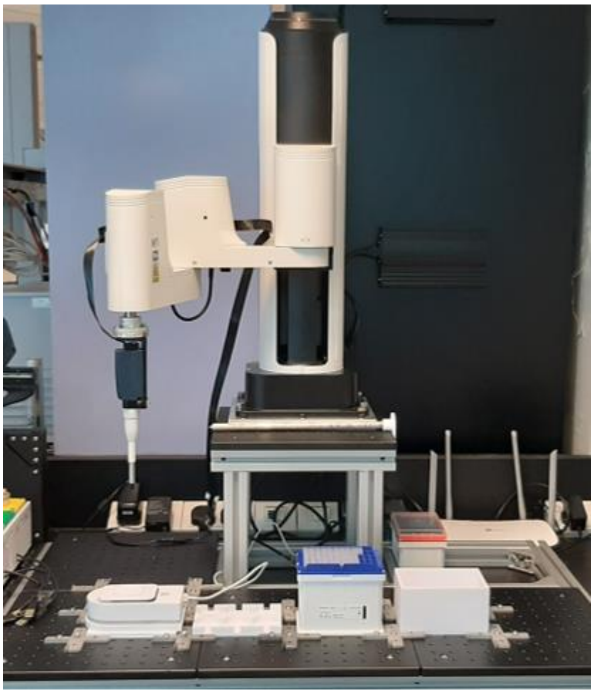

Figure S1. In-house assembled liquid handling platform. The platform consists of deck made of optical breadboards and a Sartorius rLine1000-pipette attached to a M1 Dobot SCARA arm for translation. The labware on top of the deck from left to right are: in-house built mass balance, vial holder, tip rack and trash bin.

S1.3 In-house built automated mass balance

To capture real time changes in mass during dispense/aspiration of the liquid under test, we designed and constructed a mass balance capable of streaming data to the control system. A weighing pan was secured to a cantilever TAL221 load cell (max 100g, 0.05 %FS). The voltage output of the bridge circuit was measured with a HX711 24-Bit ADC communicating with an Arduino Nano over an I2C interface. Serial communication was used to send the load cell output to the control-lab-ly Python package. The mass is recorded live at a rate of approximately five measurements per second

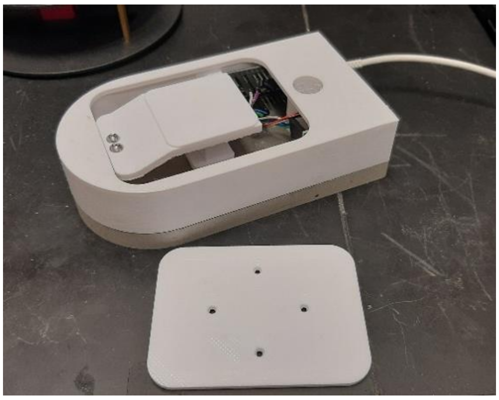

Figure S2. Automated mass balance with weighing pan removed to show TAL221 cantilever load cell, HX711 ADC, and Arduino Nano microcontroller.

S2 EXPERIMENTAL

S2.1 Gravimetric testing workflow

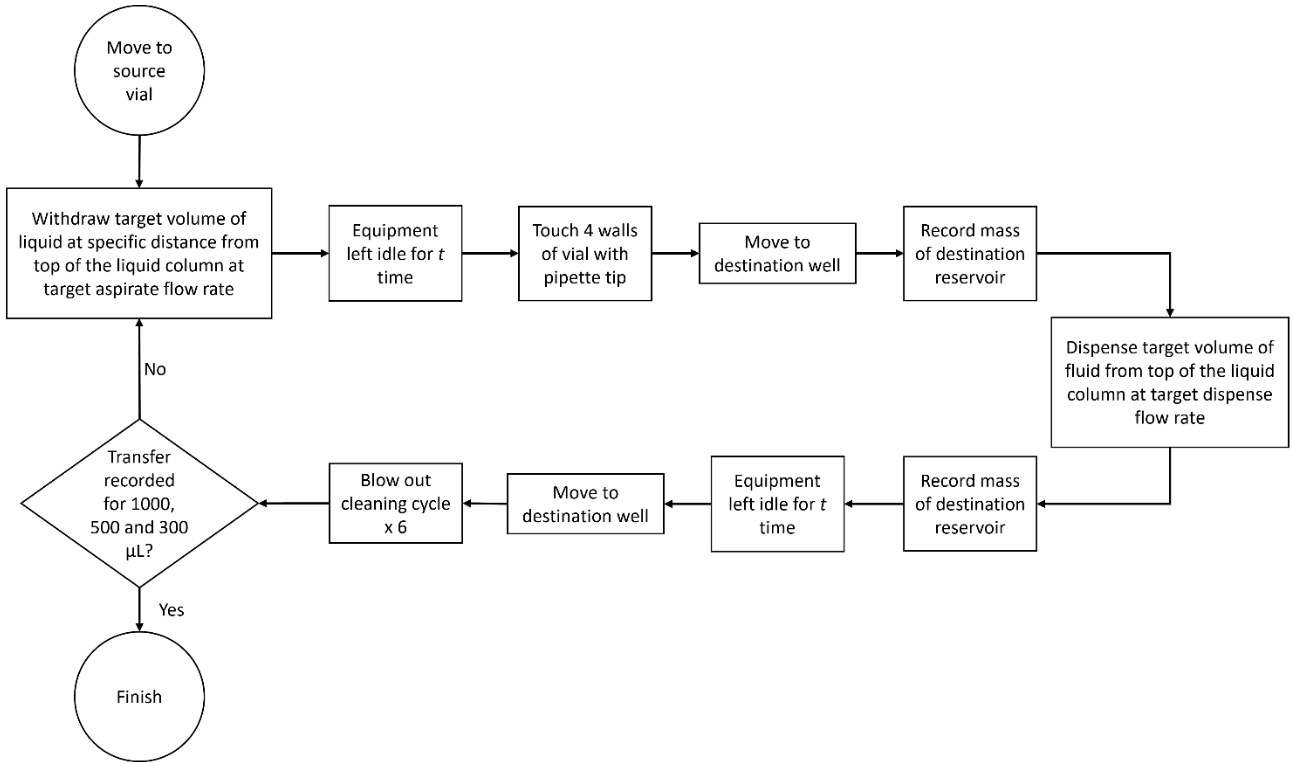

Figure S3. Diagram for the workflow for the gravimetric test of a combination of aspiration and dispense rates.

S2.2 Flow rate control for rLine1000 pipette

Not all models of electronic pipettes provide granular control on the speed of retraction and protraction of the piston. For instance, the rLine1000 is provided with six preset piston speeds that correspond to flow rates between 150 and 1120 µL/s. To achieve finer control and a wider range of flow rates, the aspiration and dispense commands can be broken down into several transfer divisions comprised of piston movement and delay intervals (Fig. S3A). The delay intervals, where the piston is idle, are interspersed between each piston movement when the command is broken down into multiple transfer divisions. The start-stop motion of the piston results in the movement of the liquid within the pipette tip at an apparent flow rate that is not included in the preset flow rates.

To achieve an average flow rate that matches a specific target flow rate (*f*) for a volume (*V*, total transferred volume), a preset flow rate (*F*) that is greater than the target flow rate is used in combination with an appropriate time delay (*T*) (Fig. S3B). Note in the equation below that it is not possible to reach a target flow rate using a slower preset flow rate than the target (Eq. 1).

$$\frac{V}{F_i} + T = \frac{V}{f}, \qquad F_i \geq f \qquad (1)$$

To calculate the optimal combination of chosen preset flow rate (F_i, i-th preset flow rate) and number of transfer divisions (*n*, number of plunger movement actions), there are several factors that has to be considered. Namely the volume resolution (r_v, the volume transferred with each incremental motion (*step*) of the plunger), the motor step resolution (r_s, the minimum number of steps that the plunger can accurately move), and time resolution (r_t, the shortest amount of time that the pipette

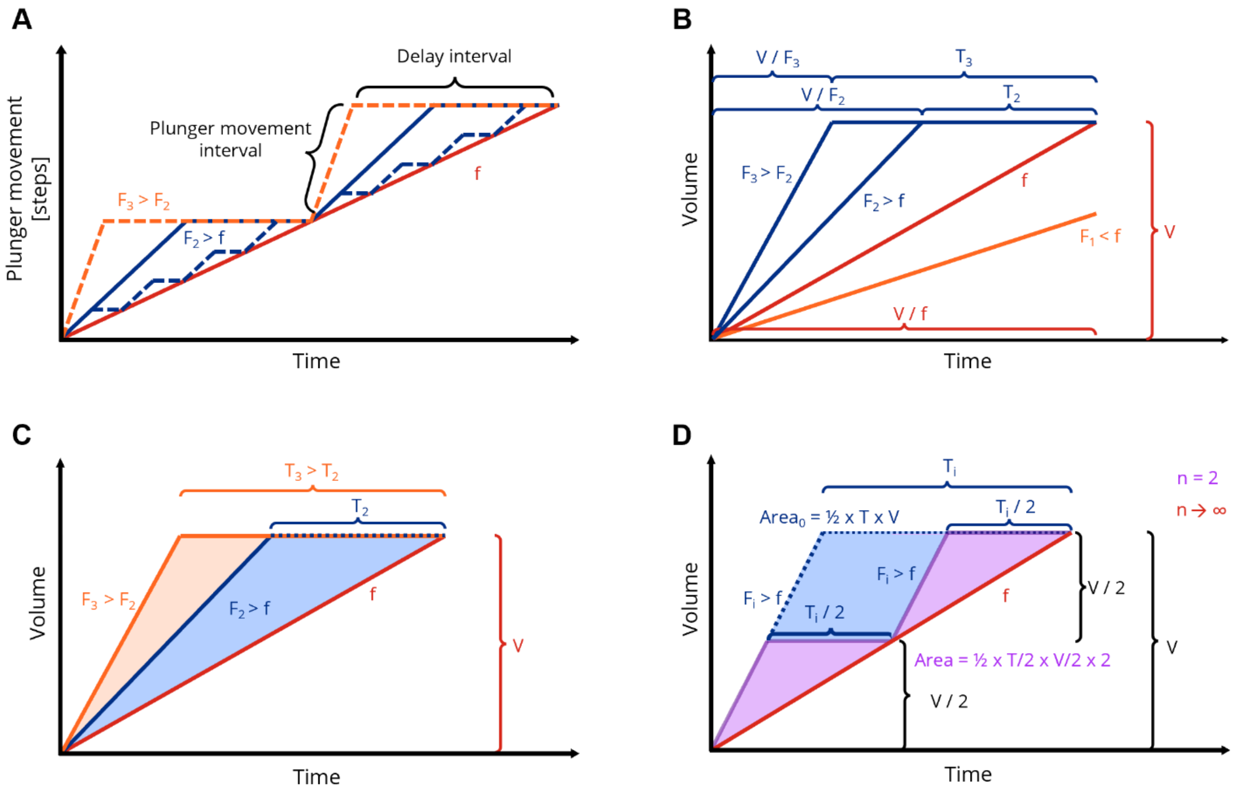

Figure S4. A) Example of different combination of plunger movement and delay intervals to achieve target flow rate (f) by using preset flow rates F2 and F3. B) A target flow rate can be achieved by using a larger preset flow rate and adding a time delay such that the average flow rate matches the target flow rate. C) For the same number of intervals, the closest preset flow rate will have the smallest deviations from the target flow rate. D) for the same chosen preset flow rate, the difference decreases with increasing number of divisions.

can respond to a given request). These restrictions place lower limits on the time delay per division (*t*) and the volume that can be transferred per transfer division (*v*). Each time delay division *t* needs to be at least equal to the time resolution of the pipette r_t (Eq. 2). Also, each volume division *v* needs to be at least equal to the minimum volume the pipette can accurately transfer, which is the product of the minimum number of steps r_s and the volume transferred with each step r_v (Eq. 3). In effect, there are upper limits on the number of transfer divisions we can break the command into due to time (L_t) and volume (L_v) restrictions (Eq. 4 and 5).

$$t = \frac{T}{n} = \frac{V}{n}\left(\frac{1}{f} - \frac{1}{F_i}\right) \geq r_t \quad (2)$$

$$v = \frac{V}{n} \geq r_v r_s \quad (3)$$

$$n \leq \frac{V}{r_t}\left(\frac{1}{f} - \frac{1}{F_i}\right) = L_t \quad (4)$$

$$n \leq \frac{V}{r_v r_s} = L_s \quad (5)$$

$$1 \leq n \leq min(L_t, L_s), \qquad n \in \mathbb{N}$$

From the inequalities above, *n* is only dependent on the choice of preset flow rate F_i, while the other terms are target values (*V, f*) or device limitations (r_t, r_s, r_v). There are two heuristics to follow in order to obtain the optimal combination of preset flow rate and number of divisions. First, for the same number of divisions, the closest preset flow rate that is greater than the target flow rate will give the smallest difference (Fig. S3C). Second, for the same chosen preset flow rate, the difference decreases with increasing number of transfer divisions. In the limit (i.e. infinite number of steps and infinitesimal delay intervals), the piston reaches constant motion at the desired piston speed and the jagged plot approaches the straight-line plot (Fig. S3D). However, this is bounded by physical and practical restrictions, which are the minimum volume that the pipette can accurately transfer, and the time taken to communicate with the pipette.

Using the heuristic that for the same chosen preset flow rate the deviation from the target flow rate decreases with increasing number of transfer divisions, we find the greatest number of divisions *n* for each preset flow rate F_i. Then after the areal difference from the straight-line plot is calculated as follows:

$$Area(n, F_i) = \frac{1}{2n}(T)(V) = \frac{1}{2n}\left(V\left(\frac{1}{f} - \frac{1}{F_i}\right)\right)(V) = \frac{V^2}{2n}\left(\frac{1}{f} - \frac{1}{F_i}\right) \quad (6)$$

Finally, the optimal combination of chosen preset flow rate and number of divisions is selected by choosing the pair that gives the smallest areal difference.

For the rLine1000, the capacity is 1000 [µL] (i.e. $V \leq 1000$) and the preset flow rates are 150, 265, 410, 650, 945 and 1120 [µL/s]. The stated values for volume resolution r_v and motor step resolution r_s provided by the manufacturer are 2.5 µL and 10 steps respectively. The time resolution r_t is empirically determined to be approximately 1.03 s.

S2.3 Automated calculation of approximate flow rate

After picking up a new tip, the robot arm moves the pipette to the top of a vial located on top of an in-house built automated mass balance, which contains the target liquid. The pipette tip is then submerged 5 mm below the surface of the liquid and the balance is tared to zero. The system is then left idle for 10 s to establish a baseline. After, the pipette is instructed to aspirate 1000 µL of liquid using a default aspiration flow rate equal to 260 µL/s. Once the aspiration is triggered the raw mass measurements are live-processed with a Savitzky–Golay filter with a window length of 91 samples and a polynomial fitting with an order equal to 1. Simultaneously the first discrete difference of the filtered mass (*dm*) is calculated. The loop is set to break when the rolling average of the last six seconds of *dm* (window approximately equal to 30) is greater than -0.05 mg. Then, the mass recording is stopped and the pipette is moved to the top of the vial.

After, the recorded mass data is fitted with the following sigmoid equation based on the generalized logistic function:

$$\sigma_m(t) = \frac{K - A}{(1 + e^{(B(t-t_0))})^{1/v}} + A$$

Where σ_m is the mass of the fitted function in function of time (t), K and A are constants that determine the values for the upper and lower asymptotes (maximum and minimum values of the sigmoid function), B is the growth rate constant that controls the maximum rate of change of the mass per unit time, t_0 is the starting time and v determines the asymmetry of the curve. Afterwards, the flow rate of the liquid as a function of time is obtained from the derivative of the mass fit with respect to time $\left(\frac{d\sigma_m}{dt}\right)$. The flow rate is approximated by obtaining the ratio of the first discrete difference of the mass fit values divided by the first discrete difference of the recorded time. After, the time values where the flow rate is smaller than 5% of the maximum absolute flow rate are selected and the time to fully aspirate 1000 µL is calculated by the time difference of the first and last recording. Finally, the approximated flow rate is calculated by dividing 1000 by the time to fully aspirate 1000 µL.

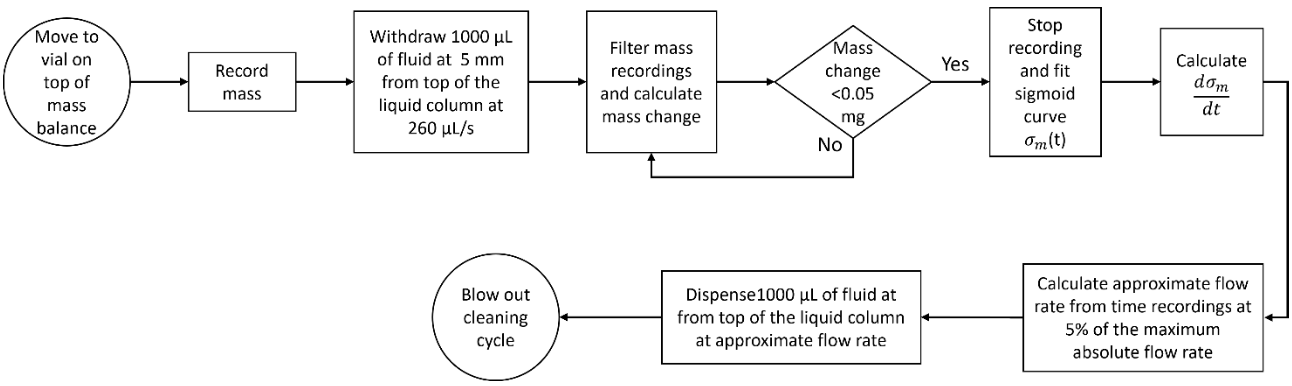

Figure S5. Diagram for the workflow for the calculation of the initial flow rate using an automated mass balance.

S3 RESULTS

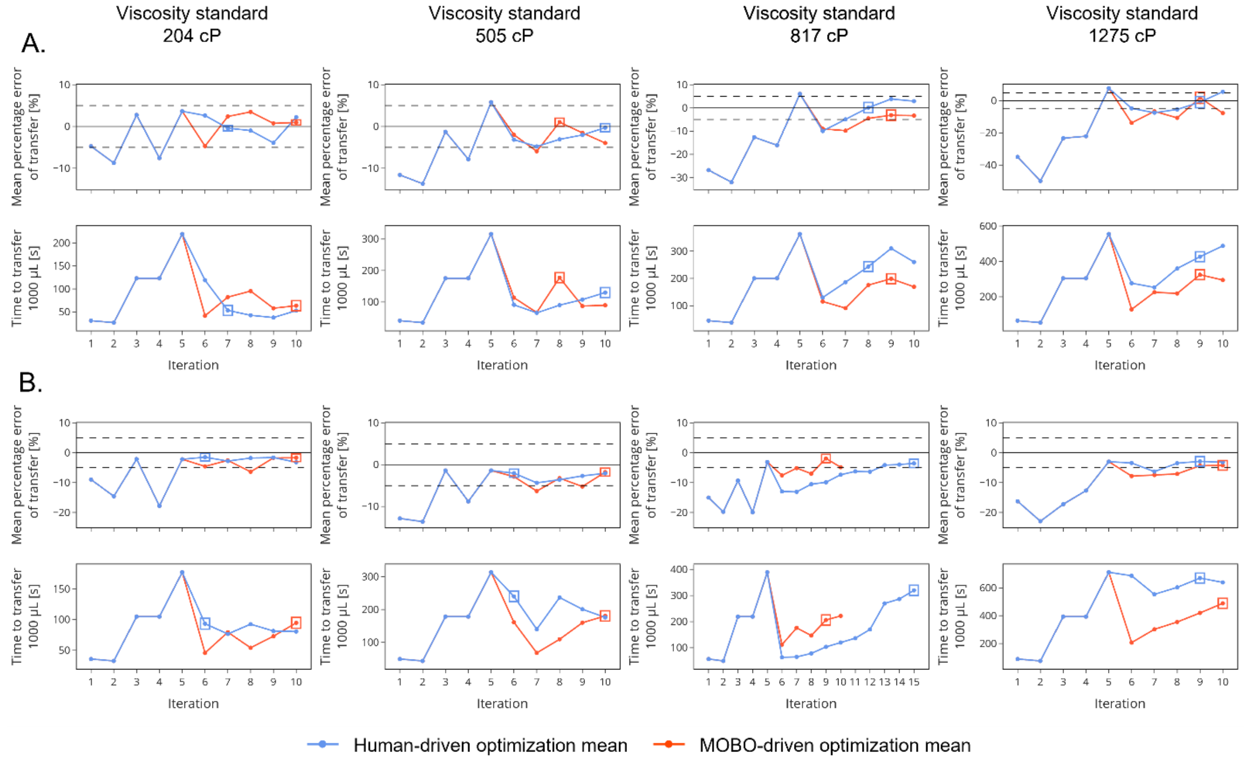

Figure S6. Optimization of liquid handling parameters for A) OT2 P1000 gen2 pipette and B) Sartorius rLine 1000 pipette.

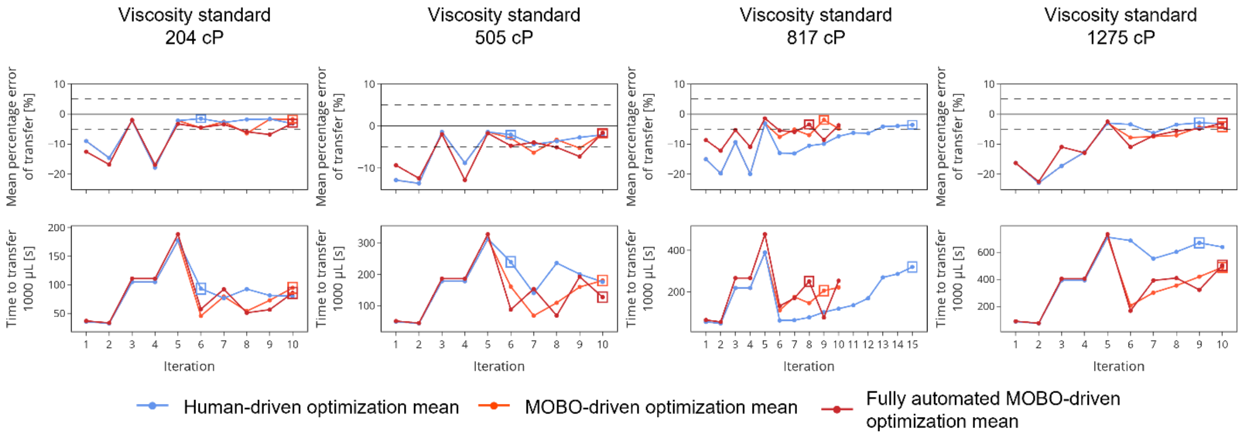

Figure S7. Optimization of liquid handling parameters for Sartorius rLine 1000 pipette using human and ML driven optimizations, including semi and full-automated protocols. The first five iterations of the MOBO and human driven optimizations overlap. When the fully automated protocol is compared with the rest it can be observed that the flow rate approximations obtained through the mass change analysis was similar to the values obtained through visual inspection.