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Automated pipetting robot for proxy high-throughput viscometry of Newtonian fluids†

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In this work, we develop a high-throughput viscometer for Newtonian fluids with viscosities between 1500 and 12 000 cP. The viscometer is developed based on an automated pipetting robot Opentrons (OT-2), with a commercial wide bore tip. The measurement protocol exploits the known characteristics of air-displacement pipettes in dispensing and gravimetrically measuring fluids of different viscosities. Specifically, by measuring the actual dispense flow rate of fluids with varying viscosities under fixed dispense conditions, we construct a calibration curve and propose a scaling analysis based on flow through an idealized pipette tip geometry. Our model can predict the viscosity of Newtonian fluids with an error of 6.5%. We also showcase the flexibility of our platform by integrating a custom-design tip for a different viscosity range.

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Introduction

High-throughput experimental approaches to discover new materials and characterize their properties have played an increasingly important role across numerous disciplines in recent years. Novel techniques for efficiently exploring and screening the design space, from automated experimentation to machine learning and artificial intelligence, have thus become a focal point of research.[1–4] Viscosity, which describes the internal friction within a moving fluid, is a key physical property of a wide range of products. Understanding the flow behavior is critical for product development in a range of sectors, including the consumer care,[5–9] oil and gas,[10–14] and pharmaceutical industries.[15–17] The ability to quickly screen for viscosity is therefore a valuable tool in accelerating the often laborious, expensive, and time-consuming research and development cycles that companies currently face.

The rotational rheometer is currently considered the gold standard for viscosity measurements. The working principle of the rheometer is to shear the fluid between two boundaries and measure the resulting displacement or torque.[18] Characterization of a single sample requires careful loading and trimming of the sample, calibration and running of the instrument, and cleaning of the geometries afterward, resulting in a lengthy and labor-intensive process. Furthermore, rheometers are costly equipment, typically with a price tag O(US$100 000).

Alternative methods to measure viscosity have been developed, emphasizing high-throughput measurements for rapid viscosity screening. For example, the capillary viscometer operates based on Hagen–Poiseuille flow and enables the determination of fluid viscosity *via* steady-state flow through a long capillary.[19–21] Both academia[21,22] and industry (*e.g.*, Viscometer-Rheometer-on-a-Chip (VROC®) by Rheosense[23]) have translated the capillary viscometer principles to a microfluidics scale, for which only small sample volumes are needed. Other efforts in this area have focused on developing microfluidic-based viscometers.[21–25] However, such methods are unable to achieve the level of throughput needed for rapid screening of large numbers of samples, for instance, in formulation development. As an illustrative example, two academic studies carried out with an industrial partner generated 224 and 338 samples, respectively, so a benchmark would be an instrument capable of characterizing hundreds of samples within a month.[26,27] Moreover, microfluidic devices typically require specialized chip fabrication and extensive cleaning, leading to high monetary costs.

Deshmukh *et al.*[28] recently proposed a high-throughput viscometer based on the automated liquid handling system, Hamilton Microlab Star. The technique analyzes mass flow behavior and pressure profiles through a pipette tip during transient flow. Routine viscosity measurements for Newtonian fluids can be carried out by comparing either the weight

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dispensed or the pressure reading to a calibration curve. The technique was demonstrated specifically on Newtonian fluids with a viscosity range of 1–5000 cP. In practice, fluids of interest to product development can have viscosities up to 10^4 cP.

In this work, we introduce a cost-effective viscometer for automated and high-throughput viscosity measurements within the range of 10^3–10^4 cP. The operating platform used is the Opentrons pipetting robot, which is more affordable relative to competing systems. Similar to Deshmukh *et al.*, our approach is gravimetric, analyzing measured mass and correlating this to the fluid viscosity, given a particular pipetting tip design. We developed a measurement protocol on the Opentrons that leverages the differing performance of air-displacement pipettes in dispensing fluids with different viscosities. For a given set of dispense conditions, we measure the actual dispense flow rate of fluids with different viscosities. A calibration curve built on scaling analysis serves as a proxy measurement to ultimately determine the viscosity of an unknown Newtonian fluid between 1500 and 12 000 cP. A single measurement can take on the order of a minute, and little human intervention is required between measurements. Therefore, viscosity characterization would no longer become the bottleneck for high-throughput studies; hundreds of samples could be measured within a week, and then the most promising candidates could be taken forward for more rigorous characterization and further study. The protocol and code developed for this study are appended (see Data availability statement), hence making the viscometer presented in this work both affordable and democratizable in that any scientific lab can choose to adopt it for viscosity measurements.

High-throughput viscometer setup

The underlying principle behind the high-throughput viscometer described in this work is to utilize the difference in the ability of the commonly-used air-displacement pipettes to handle fluids with differing viscosities. During the aspiration cycle, the piston in an air-displacement pipette is lowered to displace a volume of air according to the volume of fluid to be pipetted. It is then released to create a partial vacuum, causing the specified volume of liquid to be aspirated into the pipette tip. A constant air cushion is held between the fluid and the piston, so they are never in direct contact. This is one of the challenges of using air-displacement technology to pipette viscous fluids, as the air cushion gets stretched due to viscous forces. Our method leverages this limitation to develop a proxy viscometer.

Pipettes are typically calibrated with a reference liquid such as water, hence the performance of pipettes in handling fluids is highly dependent on the fluid properties. When a high-viscosity fluid is dispensed, the displaced air might be expelled before the fluid can be discharged from the tip. By limiting the amount of time over which fluid is dispensed, the air pressure within the pipette tip has insufficient time to equilibrate for viscous fluids, which results in a smaller volume of fluid dispensed. The differences in the volume of fluid dispensed under the same conditions can be leveraged as a proxy measurement for fluid viscosity, and an automated pipetting system can be employed to perform high-throughput measurements.

The workhorse here is the Opentrons (OT-2), a user-friendly and affordable automated liquid handling robot (see Fig. S1 in the ESI† for platform setup). The OT-2 Python protocol for these experiments is made available in the ESI.† The pipette tips used for measurements are 1000 μL wide bore tips (Thermo Scientific™ 3591). A range of general-purpose Newtonian fluid viscosity standards (Paragon Scientific) and their mixtures were used in experiments under ambient lab temperature conditions. The viscosities of the mixtures of standards were determined by separate measurements in a cone-and-plate viscometer (Brookfield DV-II+Pro) and the densities were measured by gravimetric methods. Here, the tested fluids were held in 50 mL Falcon® centrifuge tubes (VWR) supported in a custom-designed and 3D-printed holder. Custom OT-2 labware definitions are included in the Python protocol and 3D-printing file (see ESI†). We highlight that most automated liquid handling robots operate with air-displacement pipettes, hence our protocol is not limited to only the OT-2.

The protocol for a single measurement cycle is detailed in Table 1, and representative images are shown in Fig. 1(c). The protocol involves two main stages: aspiration and dispense. We highlight several points of consideration for repeatability in measurements. First, during the aspiration stage, a delay of 20s is programmed to allow for the air between the piston of the pipette and the fluid to equilibrate, which is necessary for the accurate aspiration of highly viscous fluids. Second, following aspiration of the fluid, the pipette tip is moved to four opposite edges of the reservoir to remove fluid adhered to the exterior of the tip ("touch tip"), which would otherwise drip off the tip as it

Table 1 Detailed operating protocol for measurement of fluid viscosity using Opentrons

Stage	Step	Description
Aspiration	Aspirate 375 μL fluid at set flowrate 50 μL s^{-1}	Withdraw fluid from reservoir
	Delay for 20 s	Allow for equilibration of air pressure
	Touch pipette tip to four sides of the well, performed at three different heights	Remove droplets on various locations of pipette tip exterior
	Move pipette tip to dispense plate	Aspiration stage complete
Dispense	Dispense fluid at set flowrate 50 μL s^{-1} for 5 s	Dispense fluid from pipette tip
	Touch pipette tip to four sides of the well	Remove trailing droplets on pipette tip outlet
	Move pipette tip to fluid reservoir	Dispense stage complete
	Weigh fluid	Weigh dispensed fluid

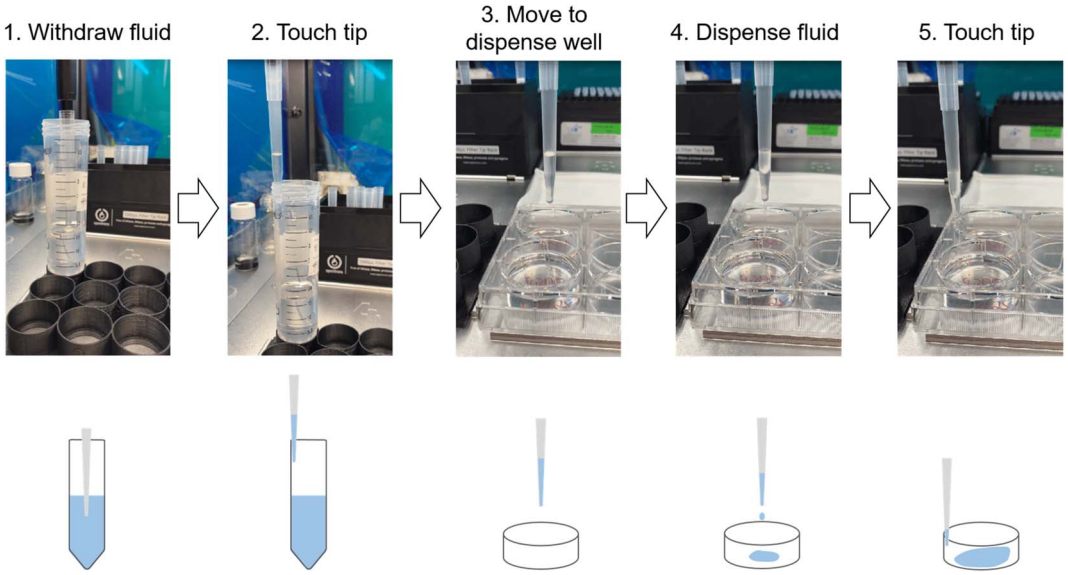

Fig. 1 Experimental protocol for the high-throughput viscometer. Series of representative images (top) and schematic (bottom) depicting the measurement protocol. During the aspiration stage, the fluid is withdrawn into the pipette tip and the tip is brought to four opposite edges of the reservoir to remove fluid along the tip exterior ("touch tip"). The tip is then moved to the dispense well (Corning 6 well plate), and the fluid is dispensed under given conditions. Touch tip is performed to remove any trailing droplets at the tip outlet.

is moved to the dispense well. This is performed at three different heights to remove fluid at different positions along the tip. Third, after dispensing the fluid, the pipette tip is again moved to four opposite edges of the well to consistently remove any trailing droplets from the tip outlet, which would otherwise not be weighed as part of the measurement. The dispense flowrate can be calculated from the measured weight based on known or measured density values and set dispense time.

A key factor that affects the accuracy of the measured dispense flowrate for viscous fluids is the adhering of fluid to the outside of the pipette tip, which occurs during the aspiration stage and also undesirably flows into the dispense plate during the dispense stage. While the "touch tip" operation after fluid aspiration reduces this effect, it is insufficient to fully remove all droplets on the exterior before the dispense stage. To account for this effect, before each flowrate measurement, we perform calibration runs in which the protocol is carried out without aspirating or dispensing any fluid. In other words, the pipette tip is dipped into the fluid reservoir and touch tip is performed as during the aspiration and dispense stages, but without any fluid being aspirated or dispensed, following which the sample-specific residue weight – a systematic error – is measured. This residue weight indicates the initial offset weight due to fluid adhered to the pipette tip exterior and is subtracted from the measured weight of dispensed fluid during analysis for proper calibration. The residue weight ranges from 0 to 4 mg.

Results and discussion

Fig. 2 shows the measured dispense flowrate, Q, as a function of set flowrate for viscosity standards with viscosities ranging from 28 cP to 4800 cP, over a time frame of 5 s as detailed in the

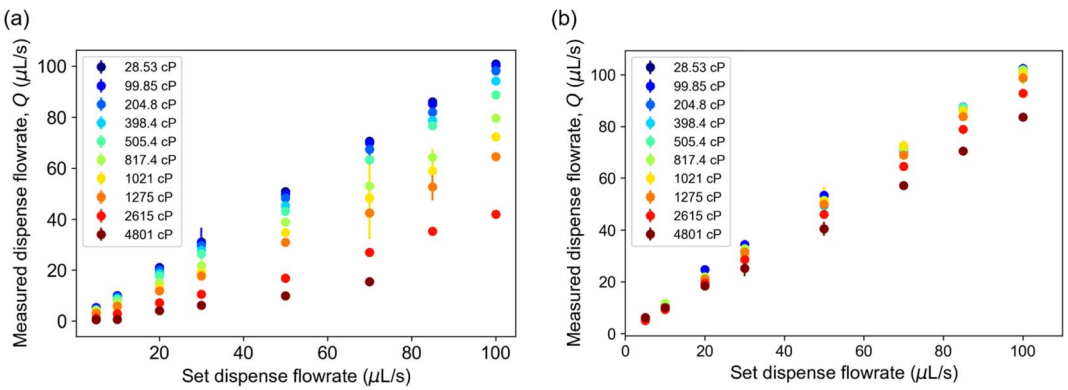

Fig. 2 Measured dispense flowrate as a function of set flowrate for viscosity standards with viscosities ranging from 28.53 cP to 4801.0 cP using (a) a standard 1000 μL pipette tip and (b) a Thermo Scientific 1000 μL wide bore tip. Fluid was dispensed over 5 s at each set flowrate. Error bars represent 95% confidence interval.

protocol in Table 1, using a standard 1000 μL pipette tip and 1000 μL wide bore tip. At low set flowrates, there is little difference in the measured dispense flowrates for the range of viscosities studied, as the compression of air between the piston and fluid occurs sufficiently slowly for pressure equilibration even with the most viscous fluids tested. At high set flowrates, the air pressure within the pipette tip is unable to fully equilibrate within the given dispense time. Therefore, at a given set flowrate, the higher the fluid viscosity, the less fluid is dispensed and the lower the measured dispense flowrate.

Compared to the wide bore tip, the standard tip can achieve greater discrimination between the lower viscosities at any given set flowrate due to the narrower orifice. However, it is unable to dispense much fluid at the higher viscosities, hence limiting its upper viscosity limit. Given that we are interested in targeting a higher viscosity range, we choose to use the wide bore tip in this work. However, we highlight that a standard tip can be used to achieve a lower viscosity detection range. A flowrate of 50 μL s^{-1} was chosen as the set dispense flowrate in the viscosity measurement protocol, given the sufficient discrimination between the measured dispense flowrates for fluids of different viscosities at this flowrate. It should be noted that the higher the selected set dispense flowrate, the greater the volume of fluid required for measurement, which can be undesirable for certain applications. Typically, high-throughput systems will prepare a larger number of samples in parallel, albeit at small volumes, so there is limited sample for testing.

The dispense flowrate for fluids of different viscosities was measured at a set dispense flowrate of 50 μL s^{-1} for 5 s, as per the measurement protocol (Fig. 3). As seen from Fig. 3(a), the higher the fluid viscosity, the lower the measured dispense flowrate, Q. For fluids with viscosities above 12 000 cP, there is inadequate delay in the aspiration stage to allow for equilibration of air pressure, which can lead to the presence of air bubbles within the pipette tip and negatively impact accuracy. For fluids with viscosities below 1500 cP, however, the dispense flowrate is equal to the set flowrate, hence the different viscosities cannot be differentiated. Therefore, with the current viscometer setup and selected parameters, the range of viscosities measurable is between 1500 cP and 12 000 cP. This extends the upper bound viscosity limit reachable with many systems – as recently highlighted by Cao *et al.*[29] it is especially challenging to develop automated measurements of high viscosity fluids. This new limit is reachable through the use of wide bore tips, with a wider orifice being more suitable for viscous fluids, and the full customizability of liquid handling parameters available by programming the OT-2.[30]

If our objective was to just obtain a calibration curve from the data, we can empirically fit a linear relationship between viscosity μ and dispense flowrate Q, as seen in Fig. 3(a). However, there is no physical basis for such an empirical fit and hence such a calibration curve is neither generalizable nor interpretable. Hence, we propose a scaling argument for the measured dispense flow rate of fluids with different viscosities. From Newton's law of viscosity,

$$\tau = \mu \frac{\mathrm{d}v}{\mathrm{d}y}$$

where τ is the shear stress and $\mathrm{d}v/\mathrm{d}y$ is the rate of shear deformation. For flow through a pipette tip with non-uniform inner diameter, we have

$$P \sim \tau \sim \mu \frac{\bar{v}}{\bar{d}}$$

where P is the applied pressure, $\bar{v}$ is the average fluid velocity through the pipette tip and $\bar{d}$ is the average tip diameter. For a constant tip geometry, the geometric factor relating the applied pressure to the pressure normal to the tip wall is constant and is thus factored out in the above equation. Based on the dispense pressure *versus* time curves reported by Deshmukh *et al.*[28] we can empirically describe the pressure profiles by power functions

$$P \sim \alpha t^{\beta},$$

where t is time and α, β are constants. Hence, for a fixed dispense time of 5s and a fixed pipette tip geometry (*i.e.*, constant $\bar{d}$), we have

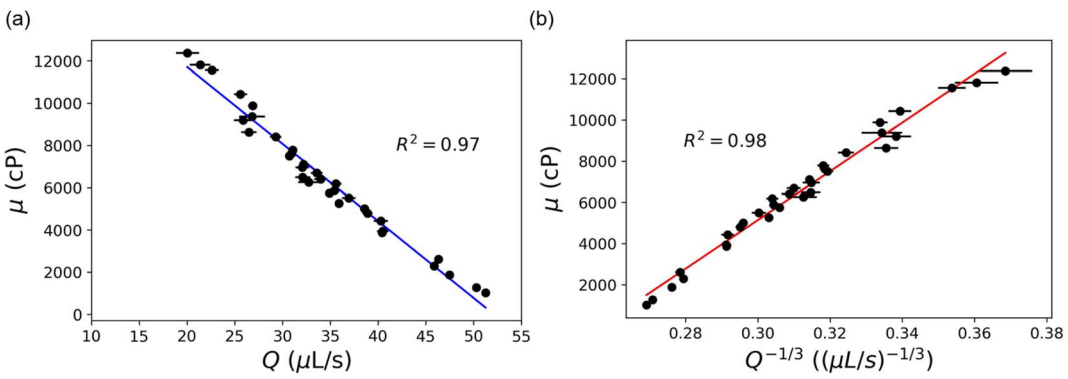

Fig. 3 Measured dispense flowrate for fluids of different viscosities at a set dispense flowrate of 50 μL s^{-1} for 5 s. (a) Viscosity, μ, as a function of measured dispense flowrate, Q, for 33 different viscosity standards. The fitted line (blue) is described by $\mu = 18\,987 - 364Q$, with a R^2 score of 0.97. (b) Viscosity as a function of $Q^{-1/3}$. The fitted line (red) is described by $\mu = -30\,236 + 117\,955Q^{-1/3}$, with a R^2 score of 0.98. Each data point is the average of 9 runs. Error bars represent 95% confidence interval.

$$\mu \sim \frac{1}{\bar{v}}. \quad (1)$$

The volumetric flow rate of fluid through the pipette tip Q is given by

$$Q = \int\int \bar{v} r \mathrm{d}r\mathrm{d}\theta \sim \bar{v} d^2.$$

For a pipette tip with diameter d of a linear profile, we have

$$d \sim \bar{v} t.$$

This leads to

$$Q \sim \bar{v}^3 t^2 \sim \bar{v}^3 \quad (2)$$

for constant t. From eqn (1) and (2) we arrive at

$$\mu \sim \frac{1}{Q^{1/3}}. \quad (3)$$

Therefore, the scaling analysis suggests that viscosity μ should scale linearly with $Q^{-1/3}$.

From Fig. 3(b), we observe a strong linear relationship between μ and $Q^{-1/3}$. The R^2 value of 0.98 shows that the data is consistent with the results of the scaling analysis. Despite the empirical linear fit having a similar R^2 value to the fit resulting from the scaling analysis, we note that the scaling analysis is based on physical arguments. Our analysis is expected to hold across viscosity ranges in which the dispense pressure profile is sufficiently described by a power function and for pipette tips with an approximately linear geometry. Testing this argument, however, is beyond the scope of this work. We examine the underlying assumptions of the analysis. First, we assume the pressure profile curves can be described by power functions. While the exact functions describing the pressure *versus* time relationship might differ, the time independence because of a set dispense time renders the consequences of this assumption insignificant on our analysis. Second, the diameter d of the pipette tip is assumed to have a linear profile and hence a linear dependence on the average velocity $\bar{v}$. For a nonlinear profile, we would expect a different dependence on $\bar{v}$, but the nonlinear dependence will be weak due to the small gradient in diameter across the tip length, for which a linear approximation is mainly valid. In addition, we note that there is a weak correlation between the fluid viscosity and error bar size, which is indicative of the protocol's limitation in accuracy as the upper viscosity range is approached. Specifically, at higher fluid viscosities, the "touch tip" process as currently implemented is unable to consistently remove trailing fluid on the tip exterior, leading to decreased precision.

For the viscosity measurement of an unknown fluid, we can therefore fit a physically meaningful calibration curve to the data presented in Fig. 3, from which the measured dispense flowrate would inform the fluid viscosity. Doing so, the residual standard error of the model is determined to be 421 cP, which is 6.5% relative to the sample mean. Hence, our model can predict viscosity of Newtonian fluids with a percentage error of 6.5%.

The protocol detailed in Table 1 allows for fast and automated measurement of fluid viscosity for Newtonian fluids in the high viscosity range up to 12 000 cP. The amount of time required to perform one measurement is approximately one minute. Furthermore, the protocol is programmed with open-source software and carried out by the OT-2, hence there is little human intervention or clean-up required between measurements. This can be compared to the traditional method for measuring viscosity using a rotational rheometer. A single viscosity measurement on a rheometer involves cleaning the plates, zeroing the gap between the plates, loading the sample, and measuring the torque at various velocities, with each step requiring active user participation. Measurement of a single fluid viscosity typically requires multiple runs, therefore the measurement of numerous samples using a rheometer can be labor intensive. This is in contrast to the automated approach for measuring fluid viscosity presented in this work.

Another key advantage of our high-throughput viscometer is the inexpensive OT-2 platform on which it is based, which is more cost-effective relative to other liquid handling robots, as well as available commercial solutions such as the automated/high-throughput Anton Paar HTR 702, GEOFF and Phil CUP/BOB robots by LABMAN automation.[29] The affordability of the OT-2 renders the viscometer developed in this work accessible to most scientific labs for which rapid viscosity screening is needed. For comparison of capabilities, however, we acknowledge that commercially available automated rheology robots can measure a range of complex rheological properties, which our setup cannot. A closer comparison would be with, for example, either of the ViscoQC or SVM viscometer series from Anton Paar, which are representative viscometer technologies at a comparable price point. These instruments require much larger samples volumes, specifically >10 mL for ViscoQC or at least 5 mL for SVM. On the other hand, our methodology requires a significantly smaller sample volume – 375 μL is needed per measurement using the protocol presented in Table 1. It is highly desirable to work at smaller scales to accelerate workflows when developing high-throughput systems, as it costs time and money (for raw materials) to prepare larger amounts of samples. A commercial robot that can work with lower sample volumes is the VROC® initium one plus. This operates based on a microfluidics technology, but it can only measure samples up to 1000 cP (with the autosampler) or 3000 cP (without the autosampler). Our presented technology is able to access a much higher viscosity range, which is relevant for many liquid formulated products, for example.[26]

Although the measurement protocol presented in this work includes a certain experimental setup and specific protocol, it should be highlighted that there is flexibility in selecting the setup and operating parameters depending on the testing requirements. For instance, the user can choose to use a larger set dispense flowrate, for which there will be larger discrepancy in actual dispense flowrates between fluids of different viscosities, to obtain greater sensitivity in measurements. As another

example, the user might incorporate the commercially available Opentrons temperature module or a custom-engineered temperature-controlled hot plate that can maintain the temperature of the fluid reservoir during aspiration, for applications that may be more temperature-sensitive.

In addition, with specialized bore tip designs integrated with the OT-2, one can tap into a different viscosity range with the same protocol. The tip geometry can be modified in several ways, including overall tip length, taper angle and length, length of the straight channel section or entrance length and tip orifice diameter. This leads to many possibilities for attaining the desired viscosity test range, or inclusion in forward models as variables to be optimized. As an example, Fig. 4 presents the technical drawings for the wide bore tip used in this study compared with a custom-designed tip geometry with a longer and narrower orifice, along with a plot of the measured dispense flowrate as a function of set flowrate for a range of viscosity standards using the custom-designed tip. With the custom-designed tip, we can achieve a large discrimination in measured flowrates for the lower viscosities, similar to the standard tip. At the same time, the custom-designed tip is able to access larger viscosity fluids compared to the standard tip, but not to the extent of the wide bore tip. As seen from Fig. 4(d), for a given set dispense flowrate and viscosity, the measured dispense flowrate with the custom-designed tip lies between the measured dispense flowrates from the standard tip and wide

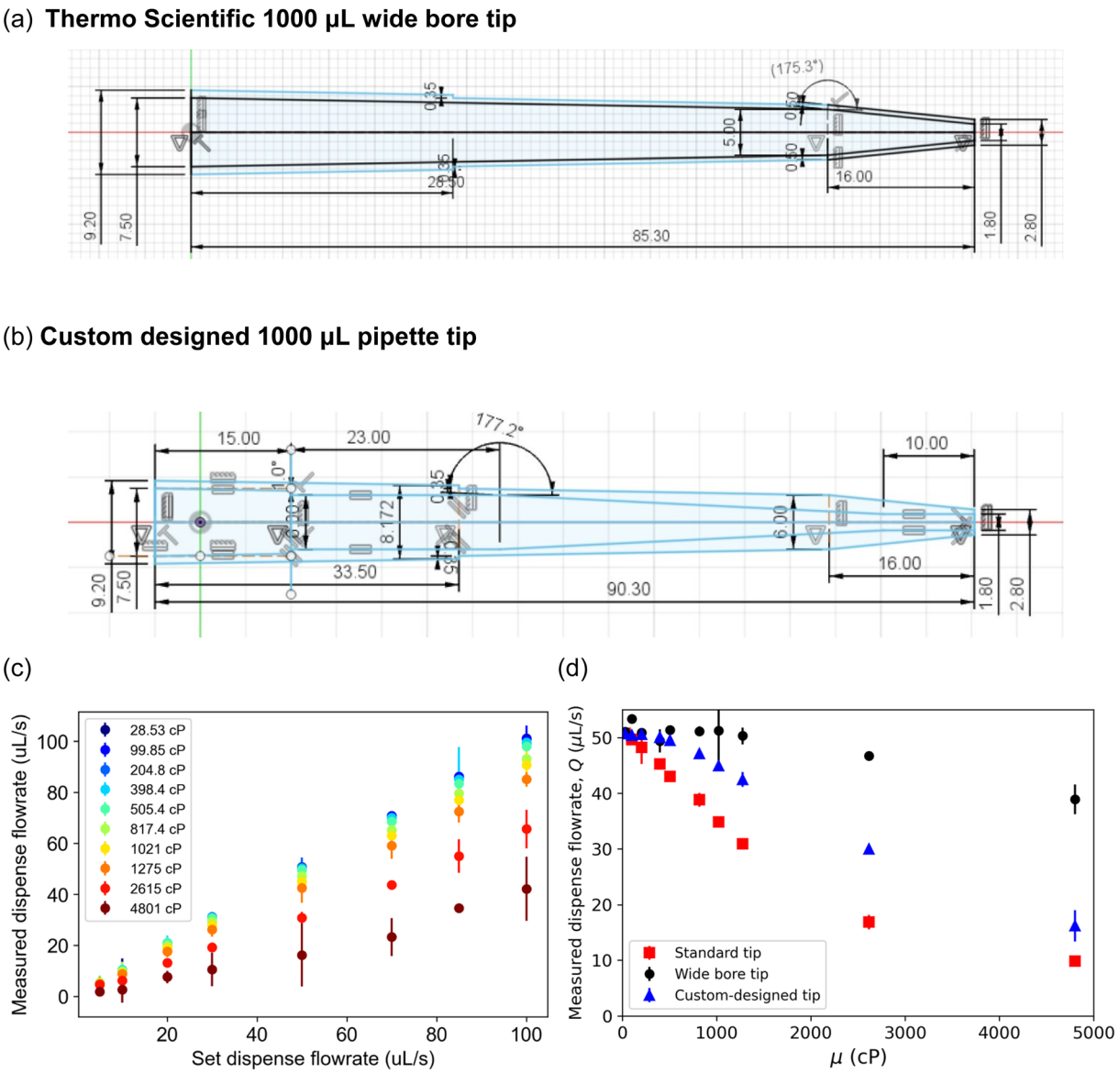

Fig. 4 Technical drawings for 1000 µL pipette tip designs. (a) Commercial wide bore tip used in the present study. (b) A custom design with a larger overall length, shorter straight channel section at the tip exit, and differing channel profile. (c) Measured dispense flowrate as a function of set flowrate for viscosity standards with viscosities ranging from 28.53 cP to 4801.0 cP using the custom-designed tip. Fluid was dispensed over 5 s at each set flowrate. (d) Measured dispense flowrate as a function of viscosity at a set dispense flowrate of 50 µL s^{-1} using the standard 1000 µL tip, Thermo Scientific 1000 µL wide bore tip and custom-designed tip. Error bars represent 95% confidence interval.

bore tip. The custom-designed tip can thus be used for fluids with an intermediate viscosity range. While Deshmukh *et al.* propose modifying the applicable viscosity range by using different pressure transducers, we introduce the possibility of fine tuning the viscosity range by using different pipette tip geometries. Therefore, while the protocol described is specific, the concept of developing a protocol for rapid viscosity measurements on the OT-2 is generalizable for different purposes.

While the current operating principle allows for the viscosity measurement of Newtonian fluids only, we can envision extending it to non-Newtonian fluids. Different set dispense flowrates can be utilized to provide different shear rates. Alternatively, different tip geometries can be customized to provide differing shear rates. Here, computational fluid dynamics (CFD) can help inform the shear rate experienced by the fluid at the tip exit. The actual dispense flowrate measured at various set flowrates would then give indication of the fluid viscosity at different shear rates, from which the rheological curve of the fluid can be constructed. Future work will focus on extending the protocol to enable viscosity measurements for general fluids.

Conclusions

In this work, we have developed an automated viscometer for the high-throughput measurements of fluid viscosity for Newtonian fluids within the range of 10^3–10^4 cP. The platform for the viscometer is the affordable and user-friendly Opentrons, an automated liquid handling robot. The operating protocol capitalizes on the differences in volume of fluid dispensed under the same conditions between fluids of different viscosities. The measured dispense flowrates for fluids with a range of viscosities at the same set dispense flowrate and time were used to construct a calibration curve, which can be employed to determine the viscosity of unknown Newtonian fluids with ~6% error. With the viscometer presented in this work, minimal human intervention is required to measure the fluid viscosity of numerous samples. Furthermore, the time involved for each measurement is much less compared to the traditional method of a rotational rheometer. The basis of the current protocol can be generalized for non-Newtonian fluids for future work.

Data availability

The code used to implement the protocol of this work and ESI† files are available at **https://github.com/Kedar-Materials-by-Design-Lab/Opentrons-ProxyViscometer**. See ESI† for more information.

Conflicts of interest

There are no conflicts to declare.

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