Tunable-Volume Handheld Pipette Utilizing a Pneumatic De-Amplification Mechanism

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TUNABLE-VOLUME HANDHELD PIPETTE UTILIZING A PNEUMATIC DE-AMPLIFICATION MECHANISM

Justin Beroz*1,2, Sheng Jiang1,2, John Lewandowski1, A. John Hart1,2
Mechanosynthesis Group
1Department of Mechanical Engineering, Massachusetts Institute of Technology
Cambridge, MA 02139
2Department of Mechanical Engineering, University of Michigan
Ann Arbor, MI 48109

ABSTRACT
We present the design, analysis, and validation of a tunable-volume handheld pipette that enables precise drawing and dispensing of ml and μl liquid volumes. The design builds upon the standard mechanism of a handheld micropipette by incorporating an elastic diaphragm that de-amplifies the volume displacement of the internal piston via compression of an entrapped air volume. The degree of de-amplification is determined by the stiffness of the elastic diaphragm and the amount of entrapped air. An analytical model of the diaphragm mechanism is derived, which guides how to achieve linear de-amplification over an extended range where leading-order nonlinear contributions are significant. In particular, nonlinearities inherent in the mechanical behavior of the diaphragm and entrapped air volume may exactly cancel one another by careful design of the pipette’s parameter constants. This linearity is a key attribute for enabling the pipette’s tunable volumetric range, as this allows diaphragms with different stiffnesses to be selectively used with a conventional linear-stepping piston mechanism. Design considerations regarding the range, accuracy, and precision of the proposed pipette are detailed based on the model. Additionally, we have constructed a handheld prototype that uses a planar latex sheet as the diaphragm. Our pipetting experiments validate the derived model and exhibit linearity between the piston stroke and drawn liquid volume. We propose that this design enables a single handheld mechanical pipette to achieve drawing and dispensing of liquids over a 1μl–10ml range (i.e., the range of the entire micropipette suite), with volumetric resolution and precision comparable to commercially available counterparts.

1. INTRODUCTION
Precision liquid handling is an essential capability in all wet laboratories that often determines the repeatability of results and the amount of chemical waste [1, 2]. While there are many types of automated precision liquid handling systems, the handheld micropipette (Fig. 1a) remains the most ubiquitous device due to its versatility and simple operation. Handheld pipettes are used for transfer and combination of µl to ml liquid volumes. As of 2006, approximately 1.5 million mechanical pipettes, 100,000 electronic pipettes, and 10 billion pipette tips are sold worldwide per year, constituting a ~$0.75 billion dollar industry with 5% annual growth [3].

A typical commercial micropipette (Fig. 1a, [4]) is held in one hand, and operated by depressing the spring-loaded push-button with the thumb. This button is mechanically attached to an internal piston, which undergoes an equal linear displacement (Fig. 1b). This piston transmits its volumetric displacement, \( V_p \), to a liquid at the tip either by direct contact (i.e., ‘positive displacement’ type) or through an intermediary air volume (i.e., ‘air cushion’ type). For both types, the volume displacement of the piston, and thereby its volumetric precision, is approximate to that of the liquid drawn into the tip. The piston stroke can be adjusted in most pipette models, typically by twisting the thumb button, which is coupled to a mechanism that translates an internal piston stop. The volume setting is indicated on a digital (or analog) volumeter.

The pervasiveness of the micropipette is, by in large, attributable to its simple mechanical design. This affords it many advantages, including cost (~$100-$500ea depending on type and brand), simple and intuitive operation, and

* Corresponding Author (jberoz@mit.edu)
straightforward maintenance. However, there are notable limitations:

1. The maximum piston stroke of a handheld pipette is ~8mm because this is the range of comfortable thumb motion. This limits the volumetric capacity per device to approximately one order of magnitude, where the absolute volume range is dictated by the piston’s cross-sectional area. As a result, typical volume ranges per micropipette are, for instance, 1–10µl, 10–100µl, 20–200µl, and 100–1000µl. Therefore, wet labs often buy micropipettes in commercially available sets that collectively cover drawing and dispensing over a larger range, such as 1µl – 1ml.

2. The positional precision of the internal piston stop is invariant for all volume settings. This leads to a comparatively higher percentage-variance for liquid volumes drawn at the lower end of the volume range of a micropipette. We estimate this to contribute ~0.2% – 0.02% variation (low end to high end) in drawn volume, presuming the precision of the piston stop is on the order of microns.

3. Drawing smaller volumes is inherently less precise due to compounding factors such as variance in liquid surface energy, pipette tip geometry, room temperature, humidity, and user technique. For instance, the calibration specifications for handheld pipettes suggest that drawn volumes at ~1µl are typically only accurate within 25% of the nominal volume [5, 6].

These limitations pose a universal “pain” for researchers and technicians requiring precision transfer of small liquid volumes or minimal waste from synthesis of dilute mixtures. This affects many routine laboratory procedures, such as titration and cell staining.

Micropipette manufacturers have sought to improve pipette performance by offering so-called electronic micropipettes that incorporate motorized actuation of the piston [7]. This improves the precision and positional resolution of piston displacement, which corresponds to moderate respective increases in accuracy (~10% at 1µl) and range (~1.5 order of magnitude) of drawn liquid volumes [6]. While these and other benefits such as programmability are favorable for some pipetting tasks, they often do not outweigh the disadvantages. More so than additional cost (> $1000ea), the added weight is a major drawback for researchers and technicians who pipette for many hours per day, which is typical in fields such as biochemistry and drug discovery. Moreover, a ‘feel’ is required for some delicate pipetting tasks – such as drawing gels and dispensing supernatant – for which a mechanical pipette is preferable.

In this paper, we propose a design for an improved handheld pipette by incorporating an intermediate elastic diaphragm between the piston and tip (Fig. 1c). This minor mechanical addition de-amplifies the volume displacement of the piston, \( V_p \), at the diaphragm, \( V_d \), via compression of the entrapped air volume. This serves to scale the pipette’s native resolution and volume capacity as a function of the amount of entrapped air and stiffness, \( k \), of the diaphragm. This de-amplification be scaled by several orders of magnitude, and therefore enables a tunable increase in the pipette’s volumetric range and precision without changing the overall design or operating procedure. To enable this, we derive a closed-form analytical model that captures the characteristics of volume transmission between the piston, \( V_p \), and diaphragm, \( V_d \). From this, we arrive at the exact mechanical behavior of the diaphragm necessary to achieve linear de-amplification. Other design considerations are also detailed, namely regarding minimization of imprecision caused by liquid head and capillary

Figure 1. (a) Typical handheld commercial micropipette [4] that aspirates liquid through a pipette tip by (b) direct action of the piston. In contrast, (c) our design de-amplifies this displacement with an intermediate elastic diaphragm. (d) Forward pipetting procedure for aspiration of an aqueous liquid volume, shown with the diaphragm mechanism.
pressure. Moreover, we have constructed a proof-of-concept prototype handheld pipette that incorporates this volume de-amplification principle, and uses a circular latex sheet used as the diaphragm (Fig. 2). Initial pipetting results agree with the model and demonstrate the importance of accounting for leading-order nonlinearities.

2. PROTOTYPE DIAPHRAGM PIPETTE

We constructed a custom-machined prototype device (Fig. 2) interfaces with commercial 1-10μl and 20-200μl pipette tips, and operates according to the conventional pipetting procedure for micropipettes (Fig. 1d). The prototype is handheld (Fig. 2b) and features a spring-loaded thumb-button mechanism similar to that found in commercial micropipettes; this includes the secondary ‘blow out’ stop for entirely clearing liquid from the pipette tip during dispensing. The piston stroke may vary from 0-350μl (i.e., 5.8mm piston diameter, 12mm max stroke), and is adjusted by changing the position of a threaded nut that acts as the piston stop (Fig. 2c). Additionally, the entrapped air volume may be adjusted by rotating the outermost casing, which translates the large piston indicated in Fig. 2a. The (red) hashed regions in the cross-section (Fig. 2c) indicate the entrapped air volume and are all in fluid communication. There is also a pressure relief (Fig. 2a) for equalizing the internal volume to the ambient air. The front of the pipette may be removed to interchange the elastic diaphragm (i.e., latex sheet). A pressure transducer line may also be installed to directly measure the pressure of the entrapped air during a piston stroke. The adjustability of this device facilitates experimental validation of all aspects of the corresponding analytical model.

3. ASPECTS OF THE DIAPHRAGM MECHANISM

Generally speaking, the advantages of incorporating an elastic diaphragm into a mechanical micropipette are:

1. The compressibility of air and elasticity of the diaphragm afford inherent precision in de-amplifying the piston’s volumetric displacement and resolution. This serves to enable higher resolution pipetting utilizing the same piston mechanism technology already developed for commercial microliter pipettes.
2. The user procedure for drawing and dispensing liquid is invariant to incorporation of the diaphragm (Fig. 1d).
3. The degree of volume de-amplification may be varied by several orders of magnitude simply by changing the amount of entrapped air, and/or diaphragm stiffness.
4. The design may incorporate several diaphragms with different stiffnesses, which may be selectively chosen to be in fluid communication with the entrapped air. This thereby extends the volumetric range of a single pipette from one to several orders of magnitude.
5. The additional mechanical complexity is minor, which, in the future, could enable a manufacturing cost that is comparable to current commercial micropipettes.

However, there is one fundamental challenge: the de-amplification of the piston displacement, \( V_p \), at the diaphragm, \( V_d \), is ideally a linear ratio. This facilitates use of a simple linear-stepping piston stop – as utilized in commercial micropipettes – for setting the piston stroke, \( V_p \). Moreover, linearity is a necessary condition for pipetting using one piston in select operation with multiple diaphragms; this is the key to achieving accurate pipetting across many orders of magnitude with one device. Unfortunately, this task is complicated by nonlinearities intrinsic to the mechanical behavior of the entrapped air and the elastic diaphragm, namely: (1) the volume and pressure of the entrapped air are inversely related; (2) elastomer materials suitable for the diaphragm (e.g., latex, PDMS) have characteristically nonlinear moduli of elasticity; (3) large deflections of the diaphragm, which are inevitable to achieve de-amplification tunable across several orders of magnitude, effectuate geometry-based nonlinearities in the diaphragm stiffness, \( k \). We suspect that this apparent difficulty accounts for why this proposed design has not been previously documented in academic or patent literature. However, the model derived in this paper shows that linear de-amplification is, in fact, possible. This is because nonlinearities arising from the stiffness of the diaphragm and compression of the air are opposite-signed, and may exactly cancel one another by careful design of the system parameters.

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\]
4. LINEAR DE-AMPLIFICATION CHARACTERISTICS

Before deriving the full model, we first focus on understanding the generalized aspects of a mechanism that transmits a linearly de-amplified displacement via compression of an intermediate fluid (Fig. 3). The labeled volume quantities are related as follows: the total volume of the transmission fluid, $V_f$, is always equivalent to some initial volume, $V_{fo}$, minus the volume displaced by the piston stroke, $V_p$, plus the volume displaced by deformation of the diaphragm, $V_d$ (Eq. 1). The corresponding differential form is Eq. 2.

$$V_f = V_{fo} - V_p + V_d$$  (1)
$$dV_f = -dV_p + dV_d$$  (2)

De-amplification of volume displacement at the diaphragm relative to the piston is represented by the quantity $dV_f/dV_p$ (Eq. 3), which we isolate by a simple rearrangement of Eq. 2. In order to interpret the right side of Eq. 3, we recognize that displacement of the piston acts on the diaphragm by means of pressure transmission, $P_f$, through the compressible fluid (i.e., the piston pushes on the fluid, which in turn pushes on the diaphragm). This is captured by chain-rule expansion of $dV_f/dV_p$ with respect to $P_f$.

$$\frac{dV_f}{dV_p} = \left(1 - \frac{dV_f}{dV_d}\right)^{-1} \left(1 - \frac{dV_f}{dP_f} \frac{dP_f}{dV_d}\right)^{-1}$$  (3)

We may now define diaphragm stiffness, $k$, as a proportional factor relating a differential change in pressure across the diaphragm, $dP_f - dP_e$, to a corresponding differential change in volume displaced by the diaphragm, $dV_d$ (Eq. 4a). $P_e$ is an arbitrary pressure applied to the diaphragm’s external side. It follows from this definition that $k$ may generally be considered a function of the absolute pressure difference across the diaphragm, $P_f - P_e$, or the total volume displaced by the diaphragm, $V_d$ (Eq. 4b). In either case, $k$ may be represented as a Taylor series expansion about $V_d = P_f - P_e = 0$. Moreover, this stiffness definition implies $k_0 > 0$.

$$k \triangleq \frac{dP_f - dP_e}{dV_d}$$  (4a)

$$k = \left\{ \begin{array}{c} \hat{k}(V_d) = k_0 + k_1 V_d + \ldots \\ \hat{k}(P_f - P_e) = k_0 + k_1(P_f - P_e) + \ldots \end{array} \right.$$  (4b)

The volumetric mechanical compressibility of the transmission fluid, $M$, is the total derivative $dV_f/dP_f$ (Eq. 5). Presumably, $M$ is negative-signed because applying a compressive (i.e., positive-signed) pressure, $P_f$, to the transmission fluid reduces its volume, $V_f$.

$$M \triangleq \frac{dV_f}{dP_f}$$  (5)

Thus, we may rewrite Eq. 3 to in terms of $k$ (Eq. 4a) and $M$ (Eq. 5), as shown in Eq. 6.

$$\frac{dV_d}{dV_p} = \left(1 - M k - M \frac{dP_e}{dV_d}\right)^{-1}$$  (6)

Provided we carefully design the mechanism to achieve linear volume de-amplification, we may identify a constant linear de-amplification ratio: $dV_f/dV_p = \text{constant}$. This term represents the characteristic performance of the mechanism, and therefore its formulation is an essential design insight. In order to focus on this term, we neglect complications arising from an external pressure disturbance (i.e., treat $dP_e = 0$). In this case, it is tractable to evaluate Eq. 6 at the unactuated state of the mechanism that we denote with subscript “0”, which corresponds to: $V_d = V_p = 0$, $V_f = V_{fo}$, $P_f = P_e = P_{atm}$ (i.e., atmospheric pressure) (Eq. 7). Thus, for all de-amplification mechanisms of this type (i.e., Fig. 3): the linear de-amplification ratio is inversely related to the product of the linear (i.e., small deformation) components of the transmission fluid’s mechanical compressibility, $M_0$, and the diaphragm stiffness, $k_0$. Notice that this is true irrespective of the composition of the transmission fluid or the material/geometry of the diaphragm.

$$\text{linear deamplification} \triangleq \frac{dV_d}{dV_p} = \left(1 + (M_0 k_0)\right)^{-1}$$  (7)

For our handheld pipette, it is sufficient to treat the ambient air used as the transmission fluid as a non-interacting (i.e., ideal) gas (Eq. 8). If this air volume does not change temperature or leak (i.e., $d(nRT) = 0$), its mechanical behavior may be described according to Eq. 9a. In this case, it is straightforward to calculate $M_0$ (Eq. 9b), and thus we have obtained the formulation for the linear de-amplification ratio for the handheld pipette. $M_0$ may be derived in a similar manner for other materials, such as a liquid with linear bulk modulus, $\beta$ (i.e., $-M_0 = \beta V_{fo}$); or a spring with linear stiffness, $k_m$ (i.e., $-M_0 = 1/k_m$), provided, of course, that $V_f$ and $V_d$ are treated as linear translations rather than volume displacements in this latter case.

$$P_f V_f = nRT$$  (8)
$$P_f V_f = P_{atm} V_{fo}$$  (9a)
$$M_0 = \left| \frac{dV_d}{dP_f} \right|_0 = -P_{atm}^{-1} V_{fo}$$  (9b)
Notably, Eq. 7 also motivates the design approach for our pipette. This linear de-amplification ratio may be considered as the linear coefficient in the Taylor series expansion of the function \( \dot{V}_d(V_p) \) about \( V_p = 0 \). Therefore, the pipette must be designed such that nonlinearities (i.e., higher-order terms) that arise over the course of piston displacement cancel out; and this may be accomplished by investigating the higher-order terms that append to Eq. 7.

### 5. DERIVATION OF MODEL

We now derive a closed-form analytical solution for a volume displacement of the diaphragm, \( V_d \), enacted by contributions from a piston displacement, \( V_p \), and externally applied pressure, \( P_e \). We utilize Taylor series expansion to preserve only the most significant nonlinearities (i.e., 2nd-order terms), which experimental results show to be sufficient. The derivation begins by returning to Eq. 6, which, after some manipulation, is reformulated as Eq. 10a. One may also arrive at this equation directly by considering the force equilibrium across the diaphragm. The mechanical compressibility of the air volume acting as the transmission fluid is the total derivative, \( dV/dP_p \), for an ideal gas (Eq. 8), and is shown evaluated in Eq. 10b.

\[
M^{-1}dV_f - dP_e = k dV_d 
\]

\[
M_{ideal} = \frac{dV_f}{dP_f} = -\frac{V_f^2}{nRT} + V_f \left( T^{-1} \frac{dT}{dP_f} + n^{-1} \frac{dn}{dP_f} \right) 
\]

For intended operation, we recognize an important design consideration: the air volume inside the rigid enclosure (Fig. 3) should be left in fluid communication with the ambient air until the instant of a piston displacement. This sidesteps the practical difficulty of preventing leakage of the air volume over long, potentially inoperative, periods of time. And, we may expect the difficulty of preventing leakage of the air volume over long, negative). The key characteristics of the mechanism are captured by Eq. 13. The diaphragm displacement, \( V_d \), as a function of piston displacement, \( V_p \), and external pressure disturbance, \( D \).

\[
V_d = \frac{V_f}{V_{fo}} - \int \frac{dV_f}{k_0 + k V_f} 
\]

\[
\begin{align*}
V_d &\approx C_{1,0} V_p + C_{2,0} V_p^2 + C_{3,0} D + C_{1,1} D V_p \\
C_{1,0} &= \frac{1}{1 + P_{am}^{-1} k_0 \left( f_v \right)} \\
C_{2,0} &= \frac{V_f \left( k_0^2 - \text{sgn}(V_p) P_{am} k_1 / 2 \right)}{P_{am} \left( 1 + P_{am}^{-1} f_v k_0 \right)^3} \\
C_{0,1} &= \frac{-V_{fo}}{1 + P_{am}^{-1} f_v k_0} \\
C_{0,2} &= \frac{V_f \left( 1 + \text{sgn}(D) P_{am}^{-1} f_v^2 k_1 / 2 \right)}{\left( 1 + P_{am}^{-1} f_v k_0 \right)^3} \\
C_{1,1} &= \frac{2V_{fo} k_0}{P_{am} \left( 1 + P_{am}^{-1} f_v k_0 \right)^3} 
\end{align*}
\]

The key characteristics of the mechanism are captured by Eq. 13. The diaphragm displacement, \( V_d \), is described by the \( C_{i,0} \) and \( C_{i,1} \) coefficients for the respective cases of an independent
piston stroke, \( V_p \) and independent pressure disturbance, \( D \). If both \( V_p \) and \( D \) occur concurrently, a coupling coefficient, \( C_{1,i} \), contributes to \( V_d \) as well. Notice that the desired linear volume de-amplification is described by the \( C_{1,0} \) coefficient, and is equivalent to that deduced in Eq. 7 and Eq. 9a.

6. PIPETTING PROCEDURE ACCORDING TO MODEL

In reference to the sequential pipetting steps in Fig 1d:

i. The pipette starts in the unactuated state, \( V_p = D = 0 \).

ii. A prescribed piston stroke, \( V_p \), displaces a volume \( V_{d,1} \) at the diaphragm (Eq. 14). The inside of the pipette tip remains exposed to atmospheric pressure, therefore \( D = 0 \).

\[
V_{d,1} \approx C_{1,0} V_p + C_{2,0} V_p^2
\]  

(14)

iii. The pipette tip is inserted into the liquid. At the instant of contact between the tip orifice and liquid, a volume of air, \( V_{Co} \), – typically referred to as the “air cushion” – is entrapped within the tip at atmospheric pressure, \( P_{atm} \), satisfying \( D = 0 \).

\[
V_{Co} = V_d,2
\]

(15)

The volume of liquid drawn into the tip, \( V_l \), is not exactly equivalent to \( V_{d,1} - V_{d,2} \) because the air cushion volume may change from \( V_{Co} \) to some \( V_C \) (Fig 1d). This air cushion may also be treated as an ideal gas (Eq. 8), from which follows Eq. 16 for the effect of an external pressure disturbance, \( \Delta \), on the diaphragm displacement and air cushion volume, respectively.

\[
V_l = V_{d,1} - V_{d,2} + V_C D (1 + D)^{-1}
\]

(16)

The disturbance pressure from a liquid with density, \( \rho \), and surface tension, \( \sigma \), comprises head and capillary pressures (Eq. 17). \( H \) is the mean curvature of the liquid meniscus inside the pipette tip, which may be considered approximately constant.

\[
D = D_{head} + D_{capillary}
\]

(17)

\[
D_{head} = \frac{\rho g h}{P_{atm}} , \quad D_{capillary} = \frac{\sigma H}{P_{atm}}
\]

7. ACCURACY AND PRECISION

There are two overall performance metrics for the diaphragm mechanism in the pipette. First, the linearity of volume de-amplification corresponds to the accuracy of the mechanism. Second, the insensitivity of the drawn liquid, \( V_f \), to some pressure disturbance, \( D \), corresponds to precision of the mechanism. The latter is because \( D \) is a variable quantity that may change based on liquid properties, environmental variations, and user technique. In light of Eq. 16, this implies: (1) The coefficient \( C_{2,0} \) from \( V_{d,1} \) must minimized for accurate pipetting; (2) the \( C_{0,i} \) coefficients (i.e., \( C_{0,1} \) and \( C_{0,2} \) from \( V_{d,2} \)) as well as the initial air cushion volume, \( V_{Co} \), should be minimal for precision pipetting.

By inspection of \( C_{2,0} \) (Eq. 13), we see that perfect accuracy may be achieved (i.e., \( C_{2,0} = 0 \)), provided there is a particular relationship between \( k_0 \) and \( k_i \) (Eq. 18). Notice that satisfying this relationship requires \( k_i > 0 \), which physically corresponds to a load stiffening response from the diaphragm. Fortunately, this mechanical behavior is typical for the large deflection of plate structures, such as the diaphragm, as tensile stresses in the middle plane begin to significantly contribute to load transmission (i.e., the membrane effect [8]). Over the course of a piston stroke, \( V_p \), load stiffening of the diaphragm contributes a negative-signed nonlinearity, while compression of the air volume, \( V_f \), contributes a positive-signed nonlinearity (i.e., the air stiffens as \( dV_f/dP_f \sim P_f^{-2} \)). The condition where \( C_{2,0} = 0 \) therefore corresponds to exact cancellation of these competing effects.

\[
k_i = 2k_0 P_{atm}^{-1}
\]

(18)

By examination of the \( C_{0,i} \) coefficients, we see that precision is improved for a small internal volume, \( V_{fo} \), and large linear stiffness, \( k_0 \). Notably, the linear de-amplification ratio, \( C_{1,0} \), is the only coefficient in Eq. 13 that does not have \( V_{fo} \) in the numerator. In the asymptotic limit \( V_{fo} \to 0 \), \( C_{0,i} \to 0 \) and scales as \( C_{0,1} \sim V_{fo} \), while \( C_{1,0} \to 1 \). This is encouraging because for smaller \( V_{fo} \), the magnitudes of \( C_{0,1} \) terms, which contribute to imprecision, fall away and leave the drawn liquid volume, \( V_f \), predominantly determined by \( C_{1,0} \). In this case, we can compensate with a large linear stiffness, \( k_0 \), to achieve the desired de-amplification ratio (i.e., magnitude of \( C_{1,0} \)). Moreover, for large \( k_0 \), the \( C_{0,1} \) terms scale as \( -k_0 \), which also serves to improve precision. Conveniently, this pipette design affords just enough parameter constants such that there is no fundamental over constraint towards maximizing accuracy and precision. Limitations therefore come from practical constraints, namely geometry.

\[
dC_{1,0} = C_{1,0}^2 \left[ \frac{k_0}{P_{atm}} \right] dV_{fo} + \frac{V_{fo} k_0}{P_{atm}^2} dP_{atm} + \left[ -\frac{V_{fo}}{P_{atm}} \right] dP_{atm} \right]
\]

(19a)

\[
dC_{2,0} = P_{atm}^{-1} V_{fo} C_{1,0}^2 \left[ \frac{k_0^2}{P_{atm}^2} \right] dP_{atm} + \frac{2k_0}{P_{atm}} dP_{atm} + \left( \frac{1}{2} \right) dP_{atm} \right]
\]

(19b)

Imprecision and inaccuracy may also result from variances in the parameter constants comprising the \( C_{1,0} \) coefficients (i.e., \( V_{fo}, k_0, k_i, P_{atm} \)). For brevity, we presume that the diaphragm mechanism is designed and operated so that the influence of \( D \) is approximately negligible (as detailed above), and the diaphragm stiffness terms nominally satisfy Eq. 18. In this case, the sensitivity of the diaphragm mechanism to variations of a
few percent in the parameter constants may be quantified, within 1st-order, by considering the total derivatives $dC_{1,0}$ (Eq. 19a,b).

For intended operation, we may estimate: $V_f \sim 10^{-6}$ [m$^3$], $V_{f0} \sim 10^{-8}$ [m$^3$], $k_0 \sim 10^{210}$ [Pa/m$^3$], $k_1 \sim 10^{15}$ [Pa/m$^3$], $P_{atm} \sim 10^5$ [Pa], where the differential quantities (i.e., $dV_f$, $dk_0$, $dk_1$, $dP_{atm}$) are smaller by $10^2$. From this, it follows that $dC_{1,0}/C_{1,0} \sim 10^{-2}$, where all three terms in $dC_{1,0}$ are of the same order. And, the relative contribution from variation in the 2nd-order term is: $dC_{2,0}V_f/C_{1,0} \leq 10^{-2}$, where this variation is largest in the upper range of piston displacement. Similarly, all three terms in $dC_{2,0}$ are of the same order. Therefore, variation in the parameter constants by a few percent may cause variation in drawn volumes by a few percent, with equally significant contributions from linear and nonlinear terms. Most concerning is the variation $dP_{atm}$, because this fluctuates daily based on the weather, and is therefore an imprecision that the user would have to contend with during regular operation, even provided perfect manufacturing and stability of the other parameter constants (i.e., $V_{f0}$, $k_0$, $k_1$). Future work could include the design of an adjustment or self-compensation mechanism to address this.

8. EXPERIMENTAL VALIDATION

Using our prototype device (Fig. 2), we have drawn microliters of deionized water ($\rho = 1000$kg/m$^3$) according to the procedure in Fig. 1d. We record the drawn liquid volumes with a microscope USB camera, and calculate the drawn volumes by image analysis (Fig. 4a). Figures 4b and 4c show the respective linear and nonlinear relations between the drawn liquid volume and internal piston stroke. Data point is the average of 5 drawn volumes, and the error bars represent 3 standard deviations.

Interestingly, we’ve learned from our pipetting experiments that a pre-stretch in the latex diaphragm is necessary for achieving linearity, otherwise $e_1 > 2e_0/P_{atm}$, as in Figure 4c. The diaphragms are prepared by first pre-stretching the latex sheet homogeneously and in-plane (stretch ratios given in Table 1), and then bonding the latex to a 25mm OD stainless steel washer (inset, Fig. 4b,c). Therefore, our calculated diaphragm stiffnesses are a function of the diaphragm’s dimensions (i.e., diameter and thickness), material properties, and pre-stretch. Part of our future work will be determining a closed-form solution for the diaphragm deflection (i.e., $\hat{k}(V_f)$) that incorporates the hyper-elasticity of the latex. We suspect that the pre-stretch affects the diaphragm stiffness by both introducing a pre-stress and changing the apparent elastic modulus of the latex (i.e., the elastic modulus for elastomers is characteristically nonlinear).

In the results presented, we have chosen to draw liquid volumes $>5\mu$l using a standard polypropylene tip (Eppendorf, 022492039) designed for use with micropipettes for volumes of 2–200µl. For this volume range and tip, we may treat $D \approx 0$ (Eq. 17) because: (1) $D_{\text{head}}$ negligibly influences the air cushion volume, $V_C$ (i.e., $dV_C/V_C < 10^{-10}$), as well as the diaphragm’s

![Figure 4](https://proceedings.asmedigitalcollection.asme.org/doi/proceedings/277?image=media-630)

Figure 4. (a) We use a microscope camera to record drawing water into a pipette tip using the prototype device. We pipetted with two prepared latex diaphragms to demonstrate (b) linear and (c) nonlinear relations between the drawn liquid volume and internal piston stroke.

<table>
<thead>
<tr>
<th>Diaphragm</th>
<th>diameter [mm]</th>
<th>thickness [mm]</th>
<th>stretch ratio</th>
<th>$k_0$ [Pa/m$^3$]</th>
<th>$k_1$ [Pa/m$^3$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1, Fig. 5b</td>
<td>12.70</td>
<td>0.10</td>
<td>1.10</td>
<td>1.6e11</td>
<td>5.1e17</td>
</tr>
<tr>
<td>#2, Fig. 5c</td>
<td>9.52</td>
<td>0.25</td>
<td>1.06</td>
<td>0.8e11</td>
<td>10.9e17</td>
</tr>
</tbody>
</table>

residual displacement, $V_{d2}$ (i.e., $V_{d2}/V_{d1} \leq 10^2$ for $k_0 \geq 10^{10}$); and (2) $D_{\text{capillary}} \approx 0$ because the meniscus inside the tip is approximately flat (i.e., $H \approx 0$, Fig. 4a). The model therefore
reduces to Eq. 20, which facilitates a straightforward comparison with the experimental pipetting data.

\[ V_i \approx C_{1,0} V_p + C_{2,0} V_p^2 \]  

(20)

Here, the diaphragm stiffness terms, \( k_0 \) and \( k_1 \), are the only unknowns – we prescribe the piston stroke, \( V_p \), by setting the piston stop on the prototype (Fig. 2c); \( V_{fo} \) is calculated based on the geometry of the prototype’s internal air chamber; and \( P_{atm} \) is measured (100.8kPa). To validate the model, we perform three pipetting runs across the piston stroke range (0-315µl), each with a different chamber volume, \( V_{fo} \) (i.e., 0µl, 315µl, and 630µl), in Fig. 4b,c). We choose one run (e.g., \( V_{fo,a} \)) and calculate its 2nd-order polynomial fit, which provides us with linear and quadratic coefficient values to solve for the two unknown diaphragm stiffness terms (listed in Table 1). Using the same stiffness constants, we observe the model curve fitting through the two other pipetting runs (i.e., \( V_{fo,b} \) and \( V_{fo,c} \)) as a preliminary validation of the model. Moreover, \( k_1 \approx 2k_0/P_{atm} \) in Figure 5c, which agrees with the derived condition for linearity (Eq. 18). The linearity of the pipetting data in Fig. 4b results from cancellation of the nonlinear contributions from the entrapped air and diaphragm. For illustration, the contribution from each is plotted independently for \( V_{fo,a} \) in Fig. 4b, and are clearly seen to be significant.

9. CONCLUSION

We have presented the design, analysis, and preliminary experimental validation of a tunable-volume handheld pipette for drawing and dispensing liquid volumes ranging from μl to ml. This is accomplished via a diaphragm mechanism that utilizes the compressibility of an enclosed volume of air to de-amplify the volume displacement of an elastic diaphragm with respect to an internal piston stroke. We propose that this device could be manufactured using the same components and methods as current micropipettes, with only the minor mechanical addition of the elastic diaphragm. The design could include several diaphragms with different stiffnesses that may be selectively chosen to be in fluid communication with the entrapped air, thereby enabling a single handheld mechanical pipette to achieve drawing and dispensing of liquids over a 1µl–10ml range (i.e., the range of the entire micropipette suite). The diaphragms may also be designed to provide a particular de-amplification scale factors, such as “1/10”, “1/100”, “1/1000”, etc.

The presented analysis provides: (1) a general formulation for the linear de-amplification of the mechanism (Eq. 7); as well as (2) a closed-form analytical expression that captures the drawing and dispensing characteristics of the diaphragm pipette to 2nd-order (Eq. 13-16), provided the enclosed air volume is well approximated by an ideal gas. The latter reveals the load-stiffening mechanical behavior of the diaphragm necessary to achieve linearity over an extended range where these leading-order nonlinear contributions are significant (Eq. 18). Moreover, we hope the design and analysis of this diaphragm mechanism may find use in other applications as a low cost means of achieving precise linear volumetric de-amplification.

10. ACKNOWLEDGMENTS

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