# BRIEF COMMUNICATION

# THE MECHANICS OF PULLING A GLASS MICROPIPETTE

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ABSTRACT The pulling of micropipette electrodes from glass tubing has been treated as a problem of viscous flow coupled with the Newtonian dynamics of the pulling apparatus. Analytical solutions are given from which the taper profile, tip diameter, and pulling time can be obtained. The physical principles of operation of micropipette pullers are discussed.

## INTRODUCTION

The glass micropipette electrode is an essential tool in many branches of physiology. Automatic pullers for the fabrication of micropipettes from glass tubing are designed empirically, and the operation of such machines attracts much discussion, not all of it well-founded. In this paper a quantitative description of the physics of pulling a micropipette is attempted, the aim being to provide some basis for the rational design of high-performance pipette pullers.

## THEORY

In the type of puller to be considered (Livingston and Duggar, 1934) a length of glass tubing is heated at its center for a preset time and then the two ends are drawn apart by toothed wheels driven with a spring. In Fig. 1 *A* the tubing occupies the region $-\infty < \ell < +\infty$; by symmetry only positive values of $\ell$ need be treated. The temperature of the glass is a maximum at $\ell = 0$, and declines towards room temperature with increasing distance from the center. The viscosity, which is a steeply decreasing function of temperature (Jones, 1971) accordingly has a minimum value $\eta_0$ at $\ell = 0$, and increases rapidly on each side to a very large value. In the absence of precise information regarding the temperature profile, we are free to specify the viscosity profile in any convenient way. A function which is found to lead to particularly simple results is:

$$\eta = \eta(\ell) = \eta_0 \exp(\ell/\ell_0), \qquad (1)$$

where $\ell_0$ is a characteristic length related, among other things, to the width of the heating coil and the duration of the heating phase.

The spring is represented by a constant pulling force $F^*$ applied at time $t = 0$ to the end of the tubing, in series with the equivalent mass $m$ of the moving parts of the machine. At times

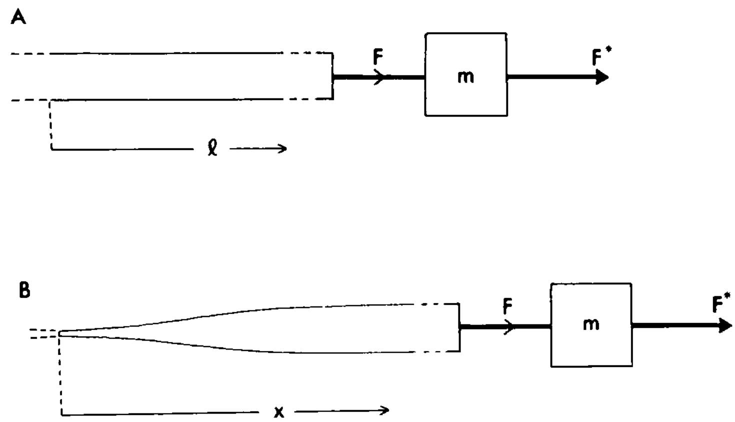


FIGURE 1 Schema of arrangement considered for simulated pulling of micropipettes. Only the right half of the tubing is shown, the continuation to the left being indicated by interrupted lines. $F^*$ is the force applied by the spring of the pipette puller, $m$ is the mass of the moving parts, and $F$ is the tensile force in the tubing. (A) At time $t = 0$. (B) At times $t > 0$.

$t > 0$ the appearance of the pipette becomes that shown in Fig. 1 *B*, where the length variable has been written $x$. The variable $x$ is to be understood as an absolute coordinate pointing to a fixed region in space, whereas $\ell$ is a Lagrangian coordinate which "follows the motion." That is, $\ell$ points to a piece of glass whose viscosity is always $\eta_0 \exp(\ell/\ell_0)$, although the glass travels in the direction of increasing $x$. The pulling process would be specified by the function $r = r(x, t)$, giving the radius $r$ at any point along the pipette's shank as a function of time. The chief simplifying assumption to be made is that pulling is so fast that the temperature, and hence viscosity, of the glass does not have time to change appreciably.

The approach will be to obtain a kinematic description of the pulling process, in which time $t$ does not appear explicitly but is replaced by a dimensionless variable $u$, defined later. The kinematic description will not involve the mass $m$ or the pulling force $F^*$. Subsequently a dynamical account will be obtained by finding $u$ as a function of time.

### *Kinematic Description*

Viscous deformation under uniaxial tension (Petrie, 1979) is governed by the relation $dA/dt = -F/3\eta$, where $A = A(\ell, t)$ is the cross-sectional area of any part of the tubing, $\eta = \eta(\ell)$ is its viscosity, and $F$ is the tensile force. In integrated form

$$A = A_0 - \frac{1}{3\eta}\int_0^t F\,dt, \tag{2}$$

where $A_0 = \frac{3}{4}\pi r_0^2$ is the cross-sectional area at $t = 0$ of tubing with external diameter $2r_0$ and internal diameter $r_0$. The dimensionless variable $u$ may now be defined; it is the ratio of the original cross-sectional area to the area remaining at the "neck" $\ell = 0$:

$$u = \frac{A_0}{A(0, t)} = \frac{3\eta_0 A_0}{3\eta_0 A_0 - \int_0^t F\,dt}. \tag{3}$$

Noting that $u$ is independent of $\ell$, we may substitute in Eq. 2 to obtain

$$\frac{A(\ell, u)}{A_0} = 1 - (1 - 1/u) \exp(-\ell/\ell_0). \tag{4}$$

Next we require a function to map the Lagrangian coordinate $\ell$ on to the absolute spatial coordinate $x$. Since deformation occurs at constant volume, $A\mathrm{d}x = A_0\mathrm{d}\ell$. Hence:

$$x(\ell, u) = \int_0^{\ell} \frac{\mathrm{d}\ell}{1 - (1 - 1/u)\exp(-\ell/\ell_0)} = \ell + \ell_0 \ln [u + (1 - u)\exp(-\ell/\ell_0)]. \tag{5}$$

It immediately follows that

$$\exp(-\ell/\ell_0) = \frac{u}{\exp(x/\ell_0) - 1 + u}. \tag{6}$$

The substitution of expression 6 into Eq. 4 eliminates $\ell$:

$$\frac{A(x, u)}{A_0} = \frac{\exp(x/\ell_0)}{\exp(x/\ell_0) - 1 + u}. \tag{7}$$

Since

$$\frac{r}{r_0} = \left[\frac{A(x, u)}{A_0}\right]^{1/2},$$

the pipette can now be drawn for any value of $u \geqslant 1$ (Fig. 2).

### *Dynamical Description*

The dimensionless variable $u$ has so far served merely as a parameter to indicate the kinematic state of the pulling process. But $u$ is a function of time and its definition (Eq. 3) includes the tensile force $F$. Knowledge of $u(t)$ will therefore lead not only to the time-dependence of pulling, but more importantly to the tensile stress in the region of the forming tip. A criterion for the separation of the tubing into two micropipettes can then be established.

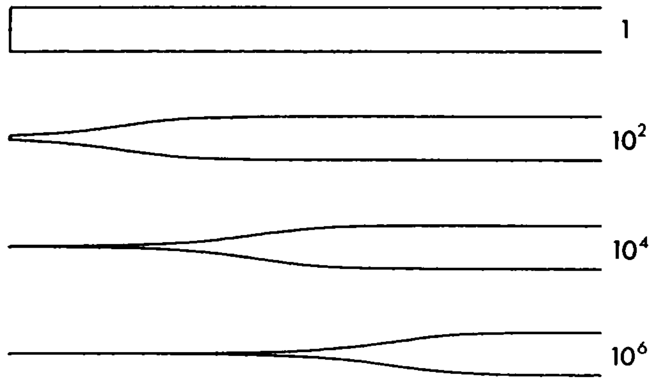


FIGURE 2 Profile views of a pipette at four stages during its formation, calculated from Eq. 7. Numbers against each drawing are values of $u$. The characteristic length $\ell_0$ was taken as 0.05 of the length shown.

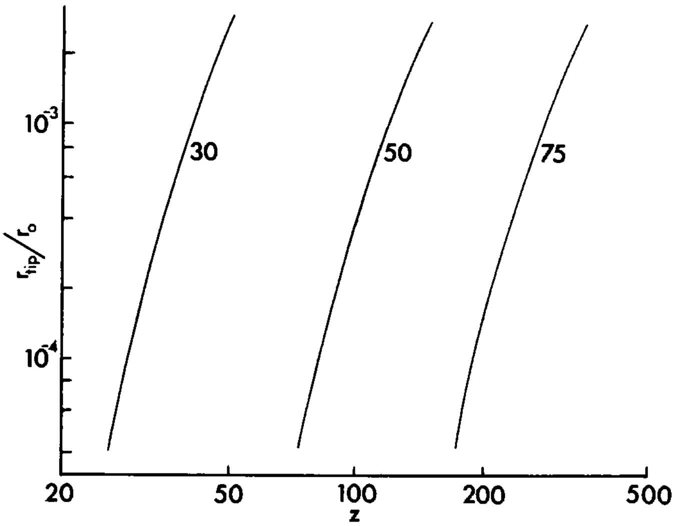


FIGURE 3 Normalized tip radius $r_{tip}/r_0$ of simulated pipettes as a function of the dimensionless variable $z = 9\eta_0^2 A_0^2/F^* \ell_0 m$. Numbers against curves are values of $A_0 S/F^*$. The curves were plotted by finding the values of $T$ (and in consequence, $u$) at which Eq. 11 is satisfied. Corresponding values of the tip radius (at $x = 0$) were then derived from Eq. 7. Note that the tip radius is a very steep function of $z$.

The dynamical description is obtained by equating the velocity of the mass to that of the glass at some large value of $x$:

$$\frac{1}{m}\int_0^t (F^* - F)\,\mathrm{d}t = \frac{F^* t}{m} - \frac{1}{m}\int_0^t F\,\mathrm{d}t = \frac{\mathrm{d}x}{\mathrm{d}t}_{x\to\infty} = \frac{\ell_0}{u}\frac{\mathrm{d}u}{\mathrm{d}t}, \qquad (8)$$

where the right hand side has been found from Eq. 5. At this point the notation is simplified by introduction of a normalized or dimensionless time variable

$$T = \frac{F^* t}{3\eta_0 A_0}.$$

Eq. 8 becomes

$$\frac{\mathrm{d}u}{\mathrm{d}T} = z(uT - u + 1), \quad T > 0; \quad u = 1, \quad T = 0, \qquad (9)$$

where the dimensionless parameter $z$ is given by

$$z = \frac{9\eta_0^2 A_0^2}{F^* \ell_0 m}.$$

The solution to Eq. 9, which may easily be verified by differentiation, is:

$$u(T) = \exp(\tfrac{1}{2}zT^2 - zT) + (\tfrac{1}{2}\pi z)^{1/2}\exp(\tfrac{1}{2}zT^2 - zT + \tfrac{1}{2}z) \times \times \{\mathrm{erf}\,[(T-1)(\tfrac{1}{2}z)^{1/2}] + \mathrm{erf}\,[(\tfrac{1}{2}z)^{1/2}]\}, \qquad (10)$$

where erf denotes the error function (Abramowitz and Stegun, 1964).

Separation into two pipettes occurs when the stress at $x = 0$ exceeds the tensile strength $S$ of glass, i.e., when

$$\frac{1}{u}\frac{du}{dT} = z\left(T - 1 + \frac{1}{u}\right) = \frac{A_0 S}{F^*}. \tag{11}$$

These results are displayed in Fig. 3 and Table I. The tip radius or diameter shows very strong dependence on most of the parameters, but only slight dependence on the radius $r_0$ of the original tubing.

## DISCUSSION

The process of pulling a micropipette has been treated as a problem in viscous fluid mechanics. The most stringent assumption to be made was that the temperature and hence viscosity of the glass do not change during the formation of the pipette. This is undoubtedly an oversimplification, but the complexities of radiative and convective heat transfer would demand an elaborate numerical approach. It seemed preferable to concentrate on the mechanical aspects of pipette formation and thereby minimize the number of physical quantities entering into the description. In the event, it proved possible to obtain analytical expressions describing the kinematics and dynamics of pipette pulling; the simplicity with which these may be evaluated provides some justification for the approach chosen.

Unconstrained viscous bodies under tension may fail either by fracture or by instability due to surface tension. Rayleigh (1892) showed that the onset of instability in a long cylinder is delayed by the viscous nature of the flow. In view of the rather high viscosity of softened glass ($\sim 10^6$ Pa s) it was assumed for the present work that fracture occurs first, and the effects of surface tension were neglected entirely. Another assumption made tacitly was that elastic strain does not contribute to any important extent.

TABLE I
PULLING TIMES AND TIP DIAMETERS OF SIMULATED MICROPIPETTES

| | $\eta_0/10^6$ Pa s | Pulling time | Tip diam |
|---|---|---|---|
| | | *ms* | *µm* |
| $F^* = 100$ N, $r_0 = 0.5$ mm | 1.1 | 31.6 | 0.020 |
| | 1.2 | 32.3 | 0.087 |
| | 1.3 | 33.2 | 0.26 |
| | 1.4 | 34.3 | 0.62 |
| | 1.5 | 35.4 | 1.25 |
| | $F^*$/N | | |
| $r_0 = 0.5$ mm, $\eta_0 = 1.3 \times 10^6$ Pa s | 80 | 41.5 | 0.047 |
| | 90 | 36.9 | 0.12 |
| | 100 | 33.2 | 0.26 |
| | 110 | 30.2 | 0.48 |
| | 120 | 27.7 | 0.81 |
| | $r_0$/mm | | |
| $F^* = 100$ N, $\eta_0 = 1.3 \times 10^6$ Pa s | 0.1 | 11.2 | 0.22 |
| | 0.25 | 15.9 | 0.26 |
| | 0.5 | 33.2 | 0.26 |
| | 1.0 | 102.1 | 0.26 |
| | 2.0 | 378 | 0.26 |

In all cases the mass $m$ was taken as 0.2 kg, the tensile strength $S$ of glass as 10 GN m$^{-2}$, and the characteristic length $\ell_0$ governing the viscosity profile as 2 mm.

The correspondence of the model with the physics of real pipette pullers may be judged from the shape of the simulated pipettes (Fig. 2) and the values given in Table I. The parameters assigned for the results of Table I have been chosen to some extent arbitrarily, but are thought to be physically reasonable. For example, the viscosity values used are in the range at which glass-blowing is done. The tensile strength $S$ is especially difficult to assign since values measured in glass fibers increase greatly with decreasing fiber diameter (Gordon, 1976). This is unfortunate, since the tip diameter of the simulated pipettes depends strongly on $S$. The value used (10 GN $m^{-2}$) is close to the theoretical maximum for glass. Most predictions of the model agree with experience: smaller tips are produced by reducing $\eta_0$ and increasing $\ell_0$, corresponding to an increase in the temperature of the heating coil and the duration of the heating phase.

Predictions not borne out by experience are that a small force $F^*$ or large mass $m$, or both, should give small tips. The discrepancy reveals much about the principles of pipette pulling. The mass $m$ has an important role in protecting the presumptive tip from the pulling force $F^*$, most of which merely acts to accelerate the mass. Thus, if $F^*$ is small and $m$ large, little force appears in the tubing as the tip is about to form, and fracture is thereby delayed until the tip becomes smaller. However, the same conditions also slow down the pulling process. In a real puller the glass will then have more time to cool. The resulting higher viscosity increases the tensile force at any given velocity of travel, and so fracture occurs at a larger tip diameter than that given by the model. Modern practice (Ensor, 1979) is aimed at increasing the speed of the pull, evidently in an attempt to approach the adiabatic conditions treated here.

The shape of the simulated micropipettes is governed principally by the viscosity profile. The particular functional form $\eta = \eta_0 \exp(\ell/\ell_0)$ was chosen because the resulting differential equation (Eq. 9) could be solved analytically. An alternative choice $\eta = \eta_0 \ell_0/(\ell_0 - \ell)$ was investigated numerically and found to give pulling times and tip diameters not very different from those of Table I. The profile of the pipettes was less realistic since the shoulder region was sharply angled rather than smoothly rounded as in Fig. 2.

There is some practical advantage in making micropipettes with short, abruptly tapering shanks (Brown and Flaming, 1977). The present analysis suggests that this would be accomplished by making $\ell_0$ small, that is by making the gradient of temperature and viscosity very steep near the middle of the tubing. The single-stage pipette puller considered here is notorious for its tendency to produce pipettes of long shallow taper; one of the advantages of the more common two-stage puller is that much of the glass is removed from the influence of the heater during the weak pull and has time to cool. Clearly this is in some respects equivalent to making $\ell_0$ small.

Chowdhury (1969) and Brown and Flaming (1977) have described pulling apparatus in which sharply tapered pipettes were produced by directing jets of gas at the heating coil just before the application of the strong pull. The authors did not speculate how the jets affected the physics of pulling, although Brown and Flaming noted that it was undesirable for the gas to impinge on the central part of the glass tubing. Evidently the principle exploited is enhanced convective cooling of the glass on each side of the tip, the heating coil acting as a barrier to deflect the gas stream away from the tip region itself. A simpler but less controllable way of achieving an abrupt taper without gas jets is to reduce $\ell_0$ directly by fitting a narrow, close-fitting heating coil. If no other modification is made to the puller, it may be found difficult to make pipettes with ultra-fine tips. Inspection of the dimensionless variables associated with Eq. 9 shows that reducing the value of $\ell_0$ has the effect of increasing $z$. A compensatory increase in the value of $m$, made by judicious addition of mass to the moving parts of the puller, should restore the original dynamics.

The present theoretical treatment has many obvious limitations but should provide a basis for further discussion. Direct experimental verification appears difficult, because some of the relevant parameters cannot easily be measured. In particular, the viscosity profile and its changes with time may have to be calculated (Holloway, 1973; Jones, 1971) from the measured or assumed temperature profile of the glass tubing.

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