gradient should be zero for the observed structure when the correct potentials are used.

To prevent excessive length in the calculation, the summation was terminated when the interatomic distance under consideration was more than a certain distance (usually 2 Å) beyond the minimum in the potential function. This cutoff was found to be important in regard to its effect on the magnitude of the packing energy. On the other hand, preliminary results indicated that the calculated packing position was not greatly affected by the error caused by series termination.

The packing method has proved useful in solving the crystallographic phase problem. The calculated packing position of the molecule can be found with sufficient accuracy to permit routine refinement of diffraction data by least squares based on structure factor, starting with the calculated packing model. The previously unknown crystal structure of dibenzoylmethane ($C_{15}H_{12}O_2$) has been solved by a preliminary packing procedure based on least squares (*6*). In this structure the molecule is in a general position; thus three rotational and three translational parameters were determined for the molecule. The molecular position found by the packing program was then refined with x-ray diffraction data. The success of the packing method led us to devise the improved steepest-descent packing program described here.

This program was tested by application to several known crystal structures of aromatic hydrocarbons. The x-ray diffraction structures of naphthalene ($C_{10}H_8$) and anthracene ($C_{14}H_{10}$) have been determined by Cruickshank (*7*) and Mason (*8*). Since the molecule has a center of symmetry, only three angles required determination. For naphthalene, the carbon positions in the calculated packing structure differed by a mean of 0.04 Å from the observed positions, with a maximum deviation of 0.08 Å; corresponding figures for anthracene were 0.08 and 0.11 Å.

The x-ray diffraction structure of phenanthrene ($C_{14}H_{10}$) has been obtained by Trotter (*9*). The molecule is in a general position, but, since the space group is polar, there are only two translational parameters in addition to the three angular parameters. The carbon positions in the calculated packing structure differed by a mean of 0.08 Å from the observed positions, with a maximum deviation of 0.13 Å.

The x-ray diffraction structure of 1,3,5-triphenylbenzene ($C_{24}H_{18}$) has been determined by Farag (*10*); the space group is again polar. Three subrotation parameters specify the rotations of the three peripheral phenyl groups, making a total of eight parameters to be adjusted. The initial packing model was assumed to be completely planar, with the *ortho* hydrogens bent back 20 deg. The packing program obtained phenyl subrotation angles of +39, −31, and +35 deg. The observed angles are +34, −27, and +24 deg, all ±2 deg. A possible explanation of the larger magnitudes of the phenyl subrotation angles found by the packing program is that no subrotation potential was used. The internal molecular energy increases as the phenyl rotation angle increases (*11*). Although such a potential parameter could have been included in the calculation, we believe that the present accuracy of the nonbonded interatomic potentials is not sufficient to refine such a parameter. The carbon positions in the calculated packing structure differed by a mean of 0.26 Å from the observed positions, with a maximum deviation of 0.56 Å. Although this agreement is not as good as those in the other tests, we believe that this is close enough to allow refinement of diffraction data by rigid body structure factor least squares.

Convergence was fairly rapid for the naphthalene, anthracene, and phenanthrene structures but slow for the 1,3,5-triphenylbenzene structure, in which case the summation limit was 1.5 Å. Experience with unknown packing structures is required for further study of convergence behavior. The usual requirement for the trial packing model, that the molecules do not penetrate each other, can be easily met by simple geometrical considerations.

Stronger intermolecular attractions, such as hydrogen bonding, may be handled by the program, provided reliable potential functions are available.

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12. Work performed in the Ames Laboratory of the AEC. This is contribution No. 1621.

28 October 1964

Constant Volume, Self-Filling Nanoliter Pipette: Construction and Calibration

Abstract. *Pipettes with volumes ranging from less than one nanoliter to 200 nanoliters can be constructed by means of a simple mechanical system and calibrated by radioisotope and fluorescence techniques. Biological fluids can be transferred with a repeatability of 1 percent by this self-filling pipette of constant volume.*

Nanoliter quantities of biological fluids can be delivered conveniently and precisely by means of a constant-volume pipette consisting of a short length of small-bore quartz tubing sealed by fusion into a long piece of soft-glass support-tubing of larger bore. When the tip of the pipette is introduced into fluid, the length of quartz tubing fills automatically and completely by capillary action. Application of positive pressure by means of a syringe delivers a known volume of fluid with a high degree of precision. This volume can be calculated from the length and inside diameter of the quartz tubing. Calibration of the volume delivered may be accomplished by either radioactive or fluorescence techniques.

Nanoliter quantities of biological fluids are obtained during studies of microscopic regions of biological organisms. These fluids are usually subjected to several kinds of analyses which require the same volume of fluid for each determination. For these and other microchemical methods it is often necessary to add diluent and other reagents to the nanoliter samples. The accuracy of the results obtained depends on the precision with which such volumes can be transferred. To meet the requirements of several microchemical methods currently in use in our laboratory, we have devised a system for the construction of pipettes with volumes ranging from less than one nanoliter to 200 nanoliters, and reproducibilities of 1 percent.

Figure 1 shows the relative sizes of the two tubes and the high quality of the seal between them. The advantages of the pipette and the ease with which it is constructed are due largely to the glass-to-quartz seal. The seal is made by heating the point at which the quartz enters the soft glass tubing and causing a small portion of this low-melting-point glass to soften and collapse around the rigid quartz. It is generally believed that, because of the different properties of the two materials, such a seal is impossible. However, with the use of tubes of such small diameters, the quartz is sufficiently strong and flexible to withstand the stresses of the shrinking support tubing. The support tubing is commercially available standard soft-glass capillary tubing (*1*). The quartz tubing, Santotube "Q," is available with inside diameters from approximately 40 μ to 90 μ (*2*). By choosing different inside diameters and lengths of this tubing, pipettes with a large range of volumes can be made.

With the exception of the first step, the entire procedure for constructing these pipettes is performed under magnification of at least × 60. The general procedure is as follows: (i) the larger tubing is drawn to a fine point by means of a commercial micropipette puller; (ii) this tip is broken back just enough to allow the quartz tubing to fit inside; (iii) the quartz is manipulated through the opening and centered between the walls of the soft glass; (iv) the seal is formed by using a microflame to heat the point at which the quartz enters the large tubing; (v) at the point on the quartz selected to give approximately the calculated volume, the flame is used to soften the quartz so that it can be pulled to a fine tip; (vi) the pipette is separated from the rest of the quartz at this tip and the pipette is complete.

The apparatus used for this procedure must hold the two pieces of tubing firmly and parallel and must provide sufficient relative motion between the two that the quartz can be centered within the larger tubing. In addition, the microburner and a microscope must be mounted so that they possess vertical and horizontal motion with respect to the pipette being constructed, and a means of separating the finished pipette from the remaining quartz must be included. To meet these specifications we assembled the mechanical system shown in Fig. 2. The various motions desired are derived from a

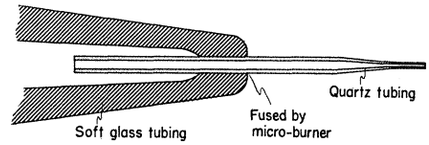

Fig. 1. Cross-section of a completed pipette indicating the relative sizes of the glass and quartz tubes and the position of the seal.

combination of two rack-and-pinion micromanipulators mounted on a single base. The original manipulator is a standard Brinkmann instrument consisting of two horizontal movements on a vertically adjustable column. Bolted to the stationary horizontal bar of this unit is a two-dimensional movement mounted vertically. The means for holding a long piece of quartz tubing is attached to the original manipulator and consists of a small aluminum block with a milled 100-μ V- slot on the top surface and a spring clamp for holding the tubing firmly in the slot. Mounted on the vertical manipulator is a simple holder for the large tubing (Fig. 2, inset *a*): a hypodermic needle, the hub of which has been fitted with a silicone rubber septum cemented in place with epoxy resin. A small hole has been made in the center of the septum so that the tubing can be pushed through the septum and into the lumen of the hypodermic. The tubing fits snugly inside a number 17 needle so that it is always parallel with the needle and is held firmly by the septum. Polyethylene tubing (PE-200) is slipped over the outside of the needle and used to connect the hypodermic needle to a syringe for providing the positive pressure needed to empty the pipette. This holder is convenient both for positioning the capillary tubing during construction and for holding the finished pipette during use; it also makes it possible for the pipette to be repeatedly inserted and removed without the necessity of tightening or loosening clamps. The microburner is a Microchemical Specialties gas-oxygen burner with a No. 27 needle waxed on to the tip to provide a smaller flame. It is mounted on a mechanical elevator which, along with a binocular dissecting microscope (× 60) is attached to the movable arm of a microscope support stand. This allows the burner and microscope to be positioned anywhere along the length of the pipette with the burner in focus in the center of the field of view. By means of hinged "axe" shown in Fig. 2 the finished pipette is separated from the remaining quartz tubing. After the quartz has been drawn to a point, the flame is moved away a

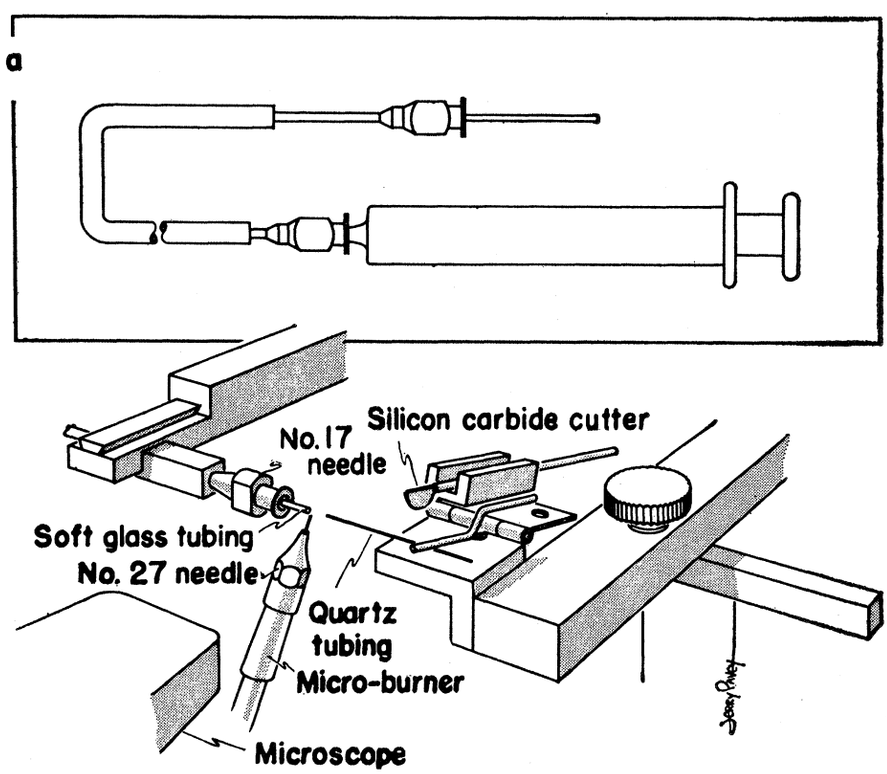

Fig. 2. The important components of the mechanical system and their relative positions. Inset *a*: details of the pipette holder-syringe assembly connected by polyethylene tubing.

Table 1. Calibration of pipettes by the fluorometric and radioisotope techniques (S.D., standard deviation).

Pipette No.	Diameter (μ) and length (mm) of quartz tubing	No. of deliveries	Volume (nl)	S.D. (nl)
	Fluorometric technique			
1	43(1.0)	9	1.86	0.03
2	43(1.4)	8	2.51	.02
3	43(2.0)	9	3.90	.04
4	90(1.25)	9	8.50	.09
	Radioisotope technique			
5	43(15.4)	3	103.2	2.55
6	90(30.7)	3	189.8	0.88

Table 2. Comparison of methods used for pipetting during calibration by fluorometry.

Pipette No.	No. of deliveries	Volume (nl)	S.D. (nl)
	Rinsed with diluent		
2	9	2.51	0.02
3	9	3.90	.04
	Complete delivery; no rinse		
2		2.43	0.03
3		3.87	.03
	Incomplete delivery; no rinse		
2		2.41	0.04
3		3.83	.06

short distance and used to heat the quartz until it can be drawn and completely separated. The pipette, with this extra quartz, is manipulated over to a brass block beneath the cutter, where it is carefully positioned so that the cutter removes the extra tubing. The cutter is a sharpened silicon-carbide chip, silver-soldered to a handle which is hinge-mounted on the aluminum block.

The pipettes may be calibrated either by fluorometric or radioisotope techniques. For fluorometric calibration, a stock solution of quinine in 0.1*N* sulfuric acid is made, and accurate standards are obtained by diluting the stock solution in distilled water to give dilutions of $1 : 10^6$ to $10 : 10^6$. Readings of these standards are taken on an Aminco-Bowman spectrophotofluorometer and a standard curve is drawn. Then the nanoliter pipettes are calibrated by pipetting from the stock quinine solution into 1 ml of distilled water and comparing these readings with the standard curve. To check reproducibility, the procedure is repeated a number of times for each pipette and the mean volume and standard deviation computed from the readings. Table 1 shows the results obtained from six of our pipettes selected at random. The first four pipettes were calibrated by the method just described; numbers 5 and 6 were calibrated by a radioisotope technique in which Na^{22} was used.

With each of the four pipettes calibrated by fluorescence, stock solution was transferred into the 1 ml of distilled water and the pipette subsequently rinsed with the solution. To prevent blowing air bubbles, the pipette was not completely emptied of the stock solution, but the amount remaining was rinsed out several times. Table 2 shows a comparison between this method of pipetting and two other methods. In the second method, the entire volume of stock solution was emptied until an air bubble was formed; the pipette was not rinsed with the solution. The apparent volume was slightly less, but the reproducibility was as good or better than with rinsing. The third method depended on incomplete delivery—that is, delivery of all the contents of the pipette except for a very small amount in the tip. No bubbles were blown, and the pipette was not rinsed with the solution. As expected, the apparent volume was less than that of either of the other two methods and the standard deviation was not as small. The amount to which this effect is noticeable depends on the length and diameter of the tip of the pipette.

As with all pipettings performed with nanoliter volumes, it is essential that the deliveries be made under microscopic observation. It is preferable that both ends of the quartz be in the field of vision so that filling and emptying can be observed. To reduce the length of the quartz required for pipettes of larger volume, it is a simple matter to form a bulb in the quartz outside the seal by heating a point with the microflame and applying pressure with the syringe.

The pipettes described can be made for volumes ranging from fractions of nanoliters to hundreds of nanoliters with reproducibilities of 1 percent over the entire range. In addition, the approximate volume of the pipettes can be quickly calculated and accurate calibration is relatively simple with the fluorescence technique outlined. Construction is simple, and with practice, as many as six to ten can be made per hour by our rather crude system. With the increased research into the minutiae of biological organisms and the resultant increase in the use of microchemical techniques these pipettes should find use in many laboratories.

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Notes

1. Flint glass capillary tubing nominally 1.0 mm outside diameter and 100 mm long; catalog No. V48302, Aloe Scientific Company.
2. Santotube "Q" quartz tubing, Monsanto Research Corporation, Nicholas Road, Dayton, Ohio.

15 December 1964

Late-Wisconsin End Moraines in Northern Canada

Abstract. *A system of end moraines nearly 2240 kilometers long has been identified by field investigation and aerial photography. It extends through northeastern Keewatin, Melville Peninsula, and Baffin Island and marks the border of a late-Wisconsin ice sheet centered over Foxe Basin and Hudson Bay 8000 or 9000 years ago.*

The late-Wisconsin glacial history of northern Canada remained largely conjectural until recently. The broad distribution of glacial features, mainly formed during deglaciation, were shown on the Glacial Map of Canada (*1*). The information on which this map is based varies considerably in reliability, with the result that large areas of the Canadian Arctic, notably Baffin Island, Melville Peninsula, and northeast Labrador-Ungava, are misleadingly shown to lack glacial features.

Our field investigations and interpretation of aerial photographs, together with study of the literature (*2–5*), show the existence of a major end-moraine system traceable, with relatively few gaps, for over 2240 km along the east coast of Baffin Island, on the west coast of Melville Peninsula, and westward across Keewatin between Committee Bay and Chantrey Inlet, with an increasing southwesterly trend to within 110 km of the head of Bathurst Inlet; thence it turns southward to Back River, beyond which it becomes indistinct (Fig. 1). An extensive system of end and lateral moraines in northeast Labrador-Ungava may be re-