

## The Accuracy and Convenience of Silicone-treated Microliter Pipettes

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The present deserved popularity of paper chromatographic techniques has resulted in the widespread use of microliter pipettes, with occasional misunderstanding of their proper usage or inherent accuracy. Existing methods of calibration (1) of pipettes of less than 100  $\mu$ l capacity are based upon the weight of mercury contained in the dry pipette. Thus, such pipettes are capable of accurate and reproducible content but not of delivery.

The advent of silicone mixtures (2, 3) that may be applied to general glassware to produce water-repellent surfaces promises great improvement in quantitative techniques, along the lines indicated by Gilbert (3). This paper enumerates several advantages which accrue to microliter chemistry by the use of water-repellent coatings.

To test the efficacy of such coatings in permitting the use of 10- $\mu$ l pipettes for delivery, two such pipettes (Microchemical Specialties No. 280B) were treated with Desicote according to the procedure outlined in the manufacturer's bulletin (2).

A calibration of the pipettes under conditions of use was accomplished using potentiometric titration of the acid delivery from each pipette. A calibrated 10-ml microburette was used for the titrations, the capillary tip dipping into the solution being titrated. The sulfuric acid delivered by the micropipettes was 13.54 *N*; titrant alkali was 0.018 *N*. The titration ratio between acid and alkali was determined for 1.00-ml samples of the diluted acid after accurate 1:100 dilution, using Normax pipette and volumetric flask. Replicate titers to pH 5.3<sup>1</sup> were 7.361, 7.358, and 7.360 ml, yielding an average of 7.360 ml  $\pm$  0.003 SE. Considering the dilution factor, the average titer thus represents a 10.00  $\mu$ l delivery of the 13.54 *N* acid. The micropipette volume may be calculated from the relation: Average volume = (average titer in ml/7.360 ml)  $\times$  10.00  $\mu$ l. The chief source of error in this calibration method is the error of dilution of the concentrated acid.

For comparison, the pipettes were also calibrated

TABLE 1  
COMPARATIVE CALIBRATION OF 10-MICROLITER PIPETTES

Pipette No.	Mercury content		Acid delivery	
	Repli-cates	Av $\pm$ SE*	Repli-cates	Av $\pm$ SE*
1	3	9.968 $\pm$ .006 $\mu$ l	2	9.961 $\pm$ .009 $\mu$ l
2	4	9.858 $\pm$ .020	2	9.841 $\pm$ .000

$$* \text{ Standard error} = \sqrt{\frac{\sum (d^2)}{N(N-1)}}$$

<sup>1</sup>The end point was arbitrarily taken as pH 5.3 to minimize carbon dioxide absorption during the titration.

with mercury by the method of Kirk (1). The results given in Table 1 show that the two methods agree to better than 0.1% and demonstrate an equality between content and delivery for silicone-treated micropipettes.

Complete delivery of the concentrated acid from the treated pipettes is indicated by the close agreement among replicate titers of single deliveries (without rinses), and by the agreement obtained between mercury content and acid delivery. In order to confirm complete delivery, the second pipette was tested for "holdup" of acid by rinsing with several portions of the titration mixture after the end point of pH 5.3 was reached. This caused a shift of less than 0.03 pH unit below the end point value. A retention of 10<sup>-5</sup>  $\mu$ l of 13.54 *N* acid on the inner surface of the pipette would result in lowering the pH of the unbuffered titration mixture by more than 0.3 pH unit. Therefore, the "holdup" of acid by this pipette was less than 10<sup>-6</sup>  $\mu$ l.

The acid delivery method of calibration may perhaps be preferred by workers who have used neither method previously. It has the advantage of simulating conditions of pipette use by the operator, thus providing a check of the operator's technique at the time of calibration. Presumably the potentiometric titration of acid delivery could be replaced by titration using the double indicator of Hawes and Skavinski (4) without great loss of accuracy for the calibration procedure.

Several precautions should be noted with regard to the use of a silicone on pipettes. The coating becomes imperfectly water-repellent unless carefully stored completely dry or completely wet so that occasional repetition of the silicone treatment is necessary. Unless it is subsequently demonstrated that the technique of silicone removal and recoating does not lead to changed pipette volumes, calibration after each cycle is necessary.

Silicone coating of microliter pipettes of the self-adjusting type (Microchemical Specialties No. 282A-283B) provides even greater convenience in their use. Certain of these pipettes, before treating with silicone, will hold several microliters of drainage liquid between the upper capillary and the bulb, which is difficult to recover by rinsing. After siliconing, such pipettes drain completely so that no visible trapping of the drainage volume occurs.

It is apparent from these results that water-repellent coating, such as that provided by Desicote, may introduce an era of accurate and convenient volume measurement for microliter chemistry as well as for macro-analysis. Such a coating provides equality between content and delivery for these 10- $\mu$ l pipettes, and presumably for larger micropipettes as well (3).

### References

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