XXXIX. A HORIZONTAL MICRO-BURETTE.

By EDWARD JOSEPH CONWAY.

*From the Department of Physiology and Biochemistry,
University College, Dublin.*

(Received December 22nd, 1933.)

IN titrations where we are restricted to determining minute quantities it is necessary to keep the final volume of fluid as low as possible, for in this way we decrease the amount of added reagent necessary to produce an observable change in concentration around the end-point and so minimise the total error. The accurate delivery of small volumes needs special burettes. With the Bang 2 ml. burette the percentage error of delivering 2 ml. is greater than the percentage error of delivering 50 ml. with the ordinary burette. Expressed as a coefficient of variation the ratio is in fact 0.11 to 0.05. Owing however to the large reduction of volume, and provided we are dealing with quantities below a certain level (about 10^{-5} equivalent), increased accuracy is secured with the micro-burette, the advantage being greater the smaller the quantity. At the same time the use of standard solutions ten times more concentrated—and hence in general more stable—is an important added advantage.

It is occasionally required to titrate quantities so minute that a burette delivering volumes of the order of 0.1 ml. is necessary. If the principle of delivering from a vertical burette is retained the delivery error alone rises to 0.45—expressed as a coefficient of variation. In Rehberg's burette [Rehberg, 1925] this delivery error appears to be much lessened by the principle of expelling the fluid by a mercury column controlled by a screw. The percentage error of delivery however can be reduced to about one-fourth the above figure by the simple expedient of delivering from a horizontally placed graduated tube. The accuracy obtained is as great as in Rehberg's burette but the ease of construction and cleaning is much greater. The percentage error of delivering 0.1 ml. with the horizontal burette here described is practically identical with the error of delivering 1 ml. with the Bang 2 ml. burette. It can therefore be substituted for the Bang burette over the whole range of micro-work. It titrates minute quantities (of the order 10^{-6} equivalent, or less) with substantially greater accuracy and permits the use of fluids ten times more concentrated and under better conditions of security from contamination by the carbon dioxide of the air or by alkali from glass surfaces—the delivered fluid having been in contact only with pyrex or paraffined walls.

Description of the burette.

Fig. 1 illustrates the structure of the burette. *A* is a piece of thermometer tubing of approximately 1 mm. bore graduated in centimetres and millimetres and placed horizontally. It is joined by a small length of pressure tubing to tube *B*. To *B* is attached the delivery tube and tap *C* and also the connecting tube and tap *D* communicating with the small container *E* on a hinged platform with support. Behind tap *D* a small window is cut in the wooden supporting

frame to allow free manipulation. All tubing except the graduated thermometer tubing (and this also if possible) should be made of pyrex glass. An advantage may be secured if the tube adjoining the graduated tube has twice or more the internal bore of this latter for then the volume of fluid in the graduated tube will be small in comparison and the emerging fluid will have been in contact only with the pyrex glass or the paraffined walls of the container. The cleansing required for a smoothly running meniscus is very easily done by detaching the graduated tube and cleaning by any of the ordinary methods. It may as a rule be cleaned sufficiently well using alcohol and ether. After cleansing it is replaced with the glass ends in contact and washed out with a

Fig. 1.

few drops of fluid from the container. The small hydrostatic pressure (5, 10 or more cm.) ensures a uniform delivery without drainage owing to the horizontal position. The rate of delivery may be found inconveniently rapid unless the delivery tip is very finely constricted. It is then liable to become blocked by any small solid particle. A simple and efficient method of adequately controlling the full delivery rate with open tap so that fine adjustments of the meniscus may be made on turning consists in attaching a glass tube to the end of the graduated section as in Fig. 1 (*F*). This dips into water below, contained in—say—a 25 ml. beaker. By sucking up some water in this tube and attaching the clip, the delivery pressure of the burette is diminished and controlled as desired.

The use of good quality thermometer tubing will ensure uniformity of bore and lengths of 25 or 50 cm. may be used as desired. With an internal bore of 1 mm. approximately 128 divisions on the burette correspond to a volume of 0.1 ml. For the container a 100 ml. reagent bottle—paraffined inside when weak alkalis are used will be found suitable.

The vertical length of the delivery tube may be varied to suit the particular fluid containers used, whether small beakers, centrifuge-tubes or the special absorbing apparatus described in a previous communication [Conway, 1933]. A standard length of about 10 cm. will suit nearly all containers. Along this delivery tube a fine pyrex tube may be attached by an elastic band or bands at the top and connected with an air-blower for stirring when centrifuge-tubes

or other such containers are used. The tip of the delivery tube may be immersed in the fluid to be titrated or immersed after the emergence of some fluid. If this method is not used a fine pyrex rod will be found suitable for removing small quantities of fluid from the tip and an amount representing a millimetre division on the graduated tube may be quite easily controlled in its emergence and transference to the titrated fluid.

The standardisation of the burette can be carried out by weighing the total of five or more deliveries of the graduated section with subsequent reference to correction tables for transferring grams to millilitres [*e.g.* those given by Stott, 1928]. A titration method may be also used. In the actual use of the burette it is however rarely required to know the volume delivered in ml., the length of the fluid column in the graduated tube corresponding on titration to a measured quantity of standard fluid being taken as the reference figure. This has the advantage of standardising at the same time the fluid in the burette.

To illustrate the action of the burette the variable error of delivering 0.1 ml. will be considered as well as a series of titrations of $10^{-3.7}$ milli-equivalents of sulphuric acid.

The variable error of delivering 0.1 ml. expressed as a coefficient of variation.

This error was investigated by a titration method. Deliveries of 0.1 ml. of *N* sulphuric acid represented by 128 mm. on the graduated tube were made into a small pyrex dish. The deliveries were successively titrated with *N*/10 carbon dioxide-free alkali from a 2 ml. standard Bang burette, a little indicator mixture of methyl red and methylene blue being added. A similar series of titrations was carried out where 1.0 ml. of *N*/10 sulphuric acid was delivered from another standard 2 ml. burette. The amount of acid titrated in both series was therefore the same and the amount of alkali necessary to change the colour of the indicator of negligible quantity owing to the strength of the solutions. The only difference affecting the variable error between the two sets is that arising from the delivery of 0.1 ml. from the horizontal burette and of 1 ml. from the Bang burette. The difference of the squares of the coefficients of variation for the two sets of titrations is therefore the difference between the squares of the coefficients for delivering these volumes. The coefficients of variation for the two sets were 0.26 and 0.25 respectively. Also the coefficient of variation for delivering 1 ml. from the Bang burette was found from 20 successive weighings to be 0.11 ± 0.02 so that it may be readily calculated that the coefficient for the horizontal burette delivery of 0.1 ml. is 0.12. Subsequent and similar series gave 0.10 and 0.15, so that 0.12 may be taken as the approximate coefficient of variation for delivering 0.1 ml. with the burette described here.

The variable error of titrating 1 ml. of 0.0002N sulphuric acid illustrating the minimum variable error of acidimetric titration.

The object of determining this error is to ascertain the least amount of alkali or acid which can be discriminated in practice by titrating with the burette. If we consider the titration of 1 ml. of *N*/10 acid delivered for example from a 1 ml. simple pyrex pipette (drawn out tube—length 17.5 cm.) the coefficient of variation in millimols of KOH is 25×10^{-5} . Where *N*/100 fluids are used the standard deviation in millimols is ten times less, and further reduction in the strength of the fluids will progressively reduce the standard deviation expressed in absolute units. We reach a point however when the standard deviation so

expressed becomes constant. This limit of standard deviation sets the limit of our discrimination and is proportional to the total buffering power of the fluid at the end multiplied by the indiscernible range here of the p_H figure. The limit is reached for the present horizontal burette when the quantity of acid titrated is in the region of 10^{-4} millimol.

Table I shows the first seven of a series of 20 titrations of 1 ml. of acid-indicator mixture of strength 0.0002 N with 0.001 N carbon dioxide-free alkali. The acid was of a kind previously described [Conway and Byrne, 1933] containing 20 % alcohol, 0.0008 % methyl red and 0.00016 % methylene blue. The standard deviation in millimols was found to be 1.7×10^{-6} . The total quantity of fluid at the end-point was 1.2 ml. contained in about 1 ml.

Table I.

No. of titration	Quantity of acid millimols	Burette reading (0.001 N KOH) cm.	Burette reading as percentage of mean reading
1	$10^{-3.7}$	25.6	101.2
2	"	25.2	99.6
3	"	25.5	100.8
4	"	25.1	99.2
5	"	25.3	100.0
6	"	25.3	100.0
7	"	25.5	100.8

The above figures give the results of the first seven titrations of a series of 20. In each case 1 ml. of 0.0002 N sulphuric acid containing 20 % alcohol, 0.0008 % methyl red, and 0.00016 % methylene blue, was titrated. The pyrex pipette used for delivering the acid delivered with a coefficient of variation of 0.16 %. The coefficient of variation of the individual titration was 0.85 %. The widest variations in the series were 1.6 and -2.0 % of the mean representing 3.2 and -4.0×10^{-6} millimol respectively. The mean error of the burette reading on a single determination was 1.85 mm. This represents 1.48×10^{-6} of a millimol and corresponds almost exactly to the error described for the Rehberg burette [Rehberg, 1925].

For titrations in atmospheric air it is not possible to diminish this figure appreciably for the above volume by titrating closer to a p_H of 7.0, for any diminution of the buffering power of the water secured thereby is more than counterbalanced by the rapidly increasing buffering power of the carbon dioxide of the air as well as by the instability of the end-point. The carbon dioxide of the air will be in equilibrium with distilled water of approximately p_H 5.6, so that the methyl red indicator has the great advantage that its point of maximum sensitivity lies in the same region.

In a carbon dioxide-free atmosphere and titrating to p_H 7.0 with the above glassware and similar fluids and volumes it is possible to decrease the standard deviation from 1.7×10^{-6} to 0.5×10^{-6} millimol KOH, provided we substitute for the indicator mixture one equally sensitive at a p_H of 7.0 and provided also our alkali is perfectly carbon dioxide-free.

The horizontal micro-burette has been found of value in the following procedures.

(1) It may be substituted for the Bang burette over the whole range of micro-titration, being more accurate for the lower ranges (10^{-3} m.eq. or less with acidimetric titration) and of the same accuracy for the higher. It possesses the added advantage of permitting the use of standard solutions ten times more concentrated. The 100 ml. storage bottle will permit the easy filling and delivery of 50 cm. length of the graduated section 250 times without any contact with the carbon dioxide of the atmosphere. The graduated section alone need be cleaned for a smoothly running meniscus.

(2) In the micro-titration of ammonia and urea as described in a previous communication [Conway, 1933] it can be substituted advantageously for the Bang 2 ml. burette. For example in the estimation of urea in 0.2 ml. of blood 1 ml. of 0.1*N* sulphuric acid may be placed in the central chamber of the absorbing unit and titrated with 0.025*N* carbon dioxide-free alkali. 50 cm. length of alkali will titrate the acid (otherwise a factor is introduced) and the calculation is simply the multiplication by 3 of the cm. of alkali on the graduated tube corresponding to the urea-ammonia. This gives the result in mg. urea per 100 ml. blood.

(3) The burette has been used for the estimation of ammonia in normal blood in conjunction with the absorbing unit described in a previous communication [Conway, 1933] and according to a procedure to be subsequently described.

(4) It has been used for the micro-titration of benzidine sulphate precipitates in centrifuge-tubes in the manner described by Cope [1931] with the Rehberg burette.

(5) It is useful in various micro-thiosulphate titrations permitting the use of comparatively concentrated standard solutions.

(6) It can be used for the very accurate delivery of small volumes of fluid of which a comparatively large quantity is available. It will deliver 0.1 ml. with from one-half to one-third the error of an Ostwald pipette, and more accurately than the ordinary analytical balance can be relied upon to weigh.

(7) It may be used as a very accurate wash-out pipette for very small volumes of blood or serum. In using it for this object water is sucked up in tube *F* (Fig. 1) to the highest level, the meniscus in the graduated tube being previously adjusted to some point near the end of the graduations. On opening the delivery tap there is now a suction pressure created which may be used to suck in a volume of blood which will be registered by the movement of the meniscus in the graduated tube. The exact volume taken in is thus measured and may then be delivered with an exact volume of wash-out water. It will be seen that this method is very convenient for small volume measurements which need not be exactly represented by tenths of a ml.

SUMMARY.

A simple micro-burette is described which can be easily assembled from pyrex thermometer tubing and graduated tubing. It delivers volumes of the order of 0.1 ml. and may be used for all micro-titrations. It is particularly suitable for titrating quantities of acid of the order 10^{-6} equivalent or less. Its error for delivering 0.1 ml. expressed as a coefficient of variation is 0.12 %. Its error for titrating 1 ml. of 0.0002*N* sulphuric acid is 1.7×10^{-6} expressed as a standard deviation in millimols of alkali.

REFERENCES.

- Conway (1933). *Biochem. J.* **27**, 430.
— and Byrne (1933). *Biochem. J.* **27**, 419.
Cope (1931). *Biochem. J.* **25**, 1183.
Rehberg (1925). *Biochem. J.* **19**, 270.
Stott (1928). *Volumetric glassware*, p. 32. (Witherby, London.)