CLIX. THE MICROMETER SYRINGE.

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(*Received November 4th, 1925.*)

IN the *Lancet* [Trevan, 1922] a piece of apparatus was described which could be used for the measurement of very small volumes of fluid, 0·01 cc. being measurable by its aid with an accuracy of less than $\pm$ 1 %.

In a slightly modified form the instrument has found considerable application, especially in the performance of the Ramon test for diphtheria antitoxin and in microchemical titrations, and it was thought that a description of the new form with some fuller details of the uses to which it has been put would be of interest. The principle of the instrument depends on the accuracy with which a fairly well-fitting piston of a glass syringe will displace a volume of fluid, the displacement being produced and measured by a Starret micrometer head such as may be obtained at any engineer's tool shop. Since the original paper was written, it has been discovered that a syringe driven by a screw had been used by both Kelvin and Lister, by the former for measuring relatively large volumes of gas in the constant pressure gas thermometer, by the latter for measuring small quantities of fluid in bacteriological research.

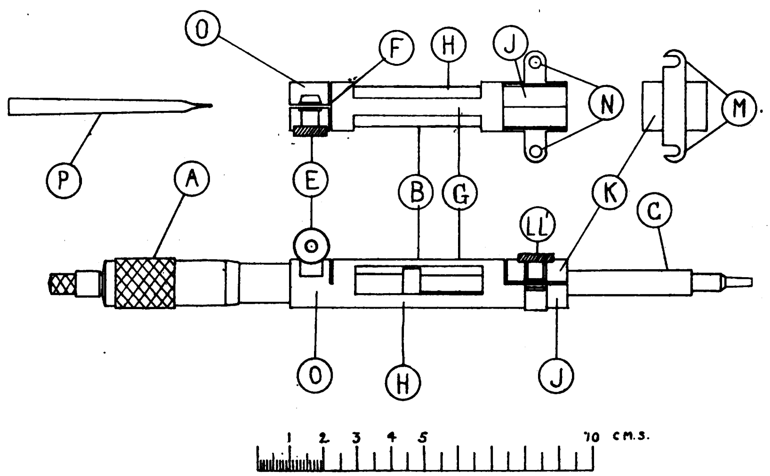

Fig. 1.

Fig. 1 shows the details. *A* is the micrometer head; *B* the holder, and *C* the syringe. The syringe illustrated is a glass "tuberculin" syringe with the piston oiled with viscous liquid paraffin, but any type of syringe can be used. The holder consists of a piece of steel tubing cut out to shape as illustrated. The end *O* is slotted as illustrated in the plan at *F* so that there is a slight give in the end sufficient for the micrometer head to be just held when it is pushed into this end of the holder. The milled head and screw *E* is provided to hold the micrometer head tightly in place. The tube is cut away leaving only the bars, *G* and *H*, the slots between allowing the head of the syringe piston to be drawn up and down. The syringe is held by the slip *JK*, the part *K* being removable. As shown in the plan, *K* is held down on *J* by the milled heads *LL'* which screw on to the sides of *J*. The lugs *M* on *K* are made with little hooks at the end which fit round the screws *N* so that *K* can be removed from *J* by merely slackening off *LL'*. *K* is lined with a piece of india-rubber pressure tubing attached with Chatterton compound, and *J* with two pieces of "fibre" arranged so as to form a "V" section groove for the syringe to lie in. The syringe is placed in the holder so that the head of the piston is just within reach when the syringe is emptied and fluid drawn up with the micrometer head withdrawn to its fullest extent. The syringe having been filled, the micrometer head is screwed down until contact is made with the piston when the instrument is ready for use. Using a micrometer head graduated in mm. in which one turn of the head corresponds to 0·5 mm. the average syringe ejects rather more than 0·01 cc. fluid for one turn. The distance through which it is necessary to move the piston to eject 0·01 cc. varies for the "tuberculin" type of syringe from 0·35 mm. to 0·55 mm., and readings of the divisions on the head can be estimated to one-tenth of a division or 0·001 mm. For some purposes it is best to choose a syringe which gives an even number of turns for 0·1 cc., *e.g.* 8, or best 10.

Calibration of syringes is best carried out by measuring the diameter of the piston of the syringe with a micrometer gauge and working out from the area of the piston the length the piston must traverse to displace 1·0 cc. Calibration by weighing mercury ejected gave figures such as the following:

Exp. 1. *Calibration with dry mercury. All-glass syringe.*

mm. reading on micrometer	Weight g.	Weight per 0·5 mm. g.
0·5	0·1267	0·1266
0·5	0·1282	0·1282
0·5	0·1272	0·1272
0·5	0·1268	0·1268
1·0	0·2545	0·1278
2·5	0·6440	0·1288
0·5	0·1270	0·1270
2·5	0·6330	0·1266
0·5	0·1270	0·1270
2·5	0·6405	0·1281
0·5	0·1270	0·1270
2·5	0·6385	0·1277
0·5	0·1285	0·1285
2·5	0·6390	0·1278
0·5	0·1265	0·1265
2·5	0·6337	0·1268
2·5	0·6395	0·1278
Mean 0·1276.	Range ±0·9 %.	Volume 0·00945 cc. = 0·5 mm.

The instrument can also be calibrated by titration. If it is filled with a strong solution of $CaCl_2$, small volumes can be ejected under the surface of water from a fine hypodermic needle and the chlorine ion titrated with $N/50$

$AgNO_3$. For the successful measurement of volumes of the order of 0·01 cc. it is best to eject the fluid under the surface of water to ensure accuracy in the separation of the last drop.

Exp. 2 *a*. *All-glass syringe.* 0·0143 cc. (= 0·5 mm. on micrometer) of a strong solution of $CaCl_2$. Titration figures; 4·42, 4·43, 4·44, 4·43, 4·44 cc. of *N*/50 $AgNO_3$. These figures do not correspond in absolute value to those in Exp. 2 *b* as different solutions of $CaCl_2$ and $AgNO_3$ were used. They are given to show the measure of agreement amongst the volumes ejected and not absolute values. It is clear that, as a good syringe does not leak back around the piston, if all the successive advancements of the piston are in agreement the absolute value must correspond to the value swept out by the advancing piston.

Exp. 2 *b*. Using 0·3 cc. syringe with metal piston with $CaCl_2$ solution. Titration figures; 6·35, 6·37, 6·38, 6·26, 6·22, 6·30, 6·27, 6·30, 6·30, 6·31 cc. *N*/50 $AgNO_3$.

Refilled syringe: 6·36, 6·40, 6·34, 6·28, 6·29, 6·34 for 2 mm.

Average 6·317 ± 0·055. Volume measured 0·004 cc.

The apparatus has been used for the measurement of 0·02 cc. of diphtheria toxin into a capillary tube for the performance of the Schick test and in the Ramon test by Glenny and Okell. A number of syringes are in daily use for this purpose by comparatively unskilled hands in these laboratories and are found to be simple and dependable.

Applications to microchemistry as a burette will be discussed at greater length in a further communication. Exp. 3 gives the results of titration of iodine with 0·096 cc. of *N*/10 thiosulphate.

Exp. 3. Approximately *N*/50 iodine. 5 cc. = 9·6 cc. *N*/100 thiosulphate, with an ordinary burette.

0·5 cc. iodine gave, in successive titration, the following figures: 0·0961, 0·0961, 0·0956, 0·0961, 0·0956, 0·0961, 0·0963, 0·0956, 0·0959, 0·0961, 0·0958, 0·0959, 0·0959, 0·0958, 0·0959 cc. as measured by the micrometer syringe using *N*/10 thiosulphate.

0·1 cc. of *N*/100 acid can be titrated using methyl red as an indicator with *N*/50 alkali with a discrepancy of at the most one in the third place. As Rehberg [1925] has pointed out, any further increase in the accuracy of micromethods of estimation can only be obtained by the use of smaller volumes. The reduction of the quantities required for titration to one-tenth of the present volumes used would be advantageous in many methods and such a reduction is certainly feasible with the apparatus described.

We think that this apparatus presents certain advantages over that of Rehberg in that the calibration of the bore of the syringe is a matter of a few seconds; there is no error of parallax, and the syringe holds sufficient fluid for many titrations or for titrations involving widely varying quantities of fluid. When titrating with acid the steel needle is replaced by a glass tube *P* drawn out to a point, and ground to fit the nozzle of the syringe. The point is covered on the outside with paraffin wax to prevent fluid running back. The final stages of the titration are carried out by allowing a small drop to collect at the tip and transferring the drop by a small glass rod (1 mm. in diameter) to the titrated fluid.

It has been used also as a gas pipette in a modified Haldane blood gas analysis apparatus. The piston is lubricated with liquid paraffin and gas leakage prevented by a layer of mercury on top of the piston, the syringe being used with the nozzle uppermost.

It can also be used as an accurate automatic pipette for large or small quantities of fluid. Exp. 4 gives a calibration by weighing dilute sulphuric acid showing the degree of agreement for a series of weighings.

Exp. 4. *Automatic pipette.* Weight of successive volumes of 1 cc. $N/10$ H_2SO_4: 1·000, 0·9999, 0·9994, 0·9994, 0·9994, 0·999, 0·9984, 1·000 g.

When used in this manner the micrometer head is screwed down until the piston rests on the plug at the bottom of the syringe, the reading on the micrometer head is then taken and the micrometer head is screwed back an amount corresponding to the volume required. Fluid is then drawn into the syringe until the head of the piston meets the micrometer plunger, and the fluid is then ejected by allowing the piston of the syringe to fall until the syringe is emptied. By joining a rubber band to the head of the syringe and passing it round the milled head *E* the syringe can be used for abstracting known volumes of blood slowly from a vein, the rubber band drawing the syringe piston slowly upwards as the micrometer is screwed out. When used as an automatic pipette, larger syringes of any volume required can be fitted into similar holders.

It may be pointed out in passing that a good hypodermic syringe may be used for the measurement of volumes without any attachment, with as much accuracy as a pipette of the same volume. For example, the piston of a tuberculin syringe can be set by eye with an error of less than $\pm$ 0·1 mm., which corresponds to 0·002 to 0·003 cc., and there is no error of parallax or of drainage as with a graduated pipette. We have also used large syringes with success for the collection of gas samples for analysis, displacing the usual cumbersome mercury bulbs, lubrication of the piston with a little water being sufficient to prevent leakage. We have also used them in place of separation funnels for small quantities of blood.

I take this opportunity of thanking Miss Boock, B.Sc., and Miss Attwood for performing many measurements for checking the accuracy of the instrument, and my mechanic, F. Gowlett, for his care and skill in making various experimental models.

Any metal worker can construct the instrument from bicycle frame tubing, but for those without facilities Messrs Burroughs Wellcome & Co., Snow Hill, London, E.C., have kindly arranged to supply the instrument with a calibrated syringe of a suitable bore.

REFERENCES.

Rehberg (1925). *Biochem. J.* **19**, 270.
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