CCCXXXIII.—*A Syringe-pipette for Precise Analytical Usage.*

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According to Ostwald–Luther (" Hand- und Hilfsbuch zur Ausführung physicochemischer Messungen," 4th edn., 1925, p. 181 *et seq.*) the limit of accuracy of pipette deliveries is about 0·5$^{0}/_{00}$, but the usual type of pipette does not reach this standard in the sizes below 5 c.c. A special form of pipette, used with the greatest care, may deliver a volume of the order of 1 c.c. with an error of 1 part in a 1000. We have carefully calibrated pipettes specially prepared for the attainment of the highest accuracy and find approximately the errors given by Ostwald–Luther.

For some time syringes have been used in this laboratory for the delivery of baryta solution in the determination of carbon dioxide (Krogh and Rehberg, *Biochem. Z.*, 1930, **225**, 177) and it had been noted that the accuracy of delivery is much better than that of pipettes when the plunger is fitted with a top stop and complete delivery is made. Trevan's micrometer syringe (*Biochem. J.*, 1925, **19**, 1111) can be used as an automatic pipette. Trevan gives the results of weighing 8 consecutive 1 c.c. deliveries from his syringe which show a mean variation representing $\pm$ 0·4 c.mm. and a maximum variation of 1·6 c.mm. From these beginnings we have developed a syringe-pipette which not only makes deliveries with unique precision, but also possesses a number of other advantages.

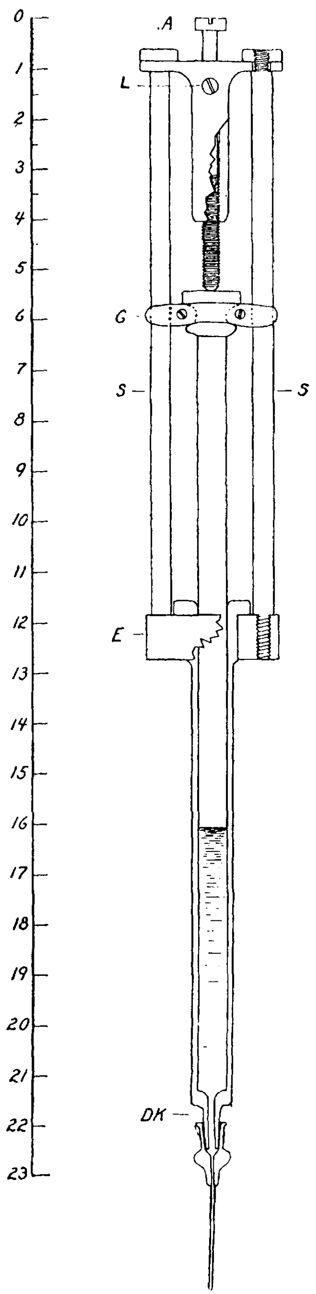
Fig. 1 shows the syringe in the filled position. The syringe itself is of glass with a solid blue glass plunger, the two being unusually closely and smoothly ground. The steel rods SS are attached to the top of the barrel by a tight-fitting ebonite collar and serve as a firm support for the top stop. The delivery of the syringe can be set to any desired fraction of the maximum capacity by means of the steel adjusting screw A, which is released or locked in position by the set screw L. The fibre guides G afford a convenient finger grip, but their chief purpose is to prevent rotation of the plunger; this is necessary because of the practical difficulty of grinding the lower end of the plunger exactly plane and 90° to the barrel and of ensuring similar exactness at contact of the plunger with the top stop. The complete syringe may be taken apart and re-assembled within a very few minutes.

Any injection needle of the Record type will fit the syringe, but we have found it advisable to seal the junction between needle and syringe with De Khotinsky or other removable cement. For most purposes blunt-pointed Krupp stainless ("Rostfreie") steel canulæ, 5—6 cm. long with external diameter of 0·80—0·90 mm., are suitable. Since the ordinary "Rostfreie" needles are made with a brass (nickel-plated outside) nib, it is well to use special needles for corrosive liquids or solutions of the noble metals. Needles made entirely of stainless steel are obtainable from Krupp on special order and these may be used for most acids, alkalis, dilute silver solutions, etc. Platinum needles will be supplied to order by the same firm. Glass needles, sealed to the syringe with De Khotinsky cement, give excellent results if properly made,* but must be handled with care.

In use, the syringe is flushed twice or more with the liquid to be delivered, driving out air bubbles completely, and is then filled slowly until the plunger firmly meets the stop. The needle is slowly withdrawn from the solution in the vertical position, shaking or contact of the needle with the wall of the container being avoided. The liquid is delivered at once with a steady motion of the plunger, the syringe always being kept in the vertical position.

Where possible, it is best to deliver with the tip of the needle immersed;

FIG. 1.



* Heavy-wall glass tubing of bore somewhat smaller than the outside diameter of the syringe tip is drawn out with a straight taper of at least 3 cm. to the delivery point, which must be cut clean and straight and have a bore at the tip between 0·3 and 0·6 mm. The end for junction with the syringe is enlarged by boring, blowing, or shaping, as is preferred, so that about 3 or 4 mm. of the needle will slip over the tip of the syringe. The junction is sealed with a liberal amount of De Khotinsky cement. It is well to polish the tip of the needle on a very smooth stone or with rouge.

quite as good results are obtained, however, if a slight amount of care is exercised when delivery is made in air. In the latter case, when the delivery is properly made, a drop of liquid will adhere to the tip of the needle; this is picked off completely by lightly touching the tip of the needle several times against the wall of the delivery vessel at an angle. Subsequent deliveries are made without necessarily flushing between each delivery; if air bubbles * appear on refilling (after delivery in air), it is an indication that the previous delivery has been made too forcibly. When not in use, the syringe may be either stored dry or filled with water. It is not necessary to grease the plunger.

We have made a large number of calibrations of these syringes by weighing successive deliveries of water. When such calibrations are made by a routine technique without special vigilance against error, weights accurate to 0·1 mg. being used on a balance similarly precise, the indicated error in the constancy of delivery is something less than ± 0·1 c.mm.

The attainable precision of the syringes was tested by weighing successive deliveries on a more delicate balance with specially calibrated weights. The most careful procedure was used and suitable precautions were taken to eliminate errors due to evaporation, change of temperature, etc. A typical result is given below:

Delivery No.	1.	2.	3.	4.
H_2O delivered, g.	1·49721	1·49721	1·49717	1·49716
Deviation from mean, mg.	+ 0·022	+ 0·022	− 0·018	− 0·028

The errors in delivering viscous liquids from the ordinary pipette are much larger than those made with liquids of viscosity similar to that of water. We have found that when the syringe-pipette is used to deliver blood serum (viscosity about twice that of water) the error is practically the same as when water is delivered.

In attaining precision of the order that we are discussing, the effects of even slight changes in temperature must be considered. Any changes in temperature of the syringe during a series of deliveries are too slight to have any effect. Changes in temperature of the room from day to day, and even during the day, presented a more serious problem, which was solved by counter-balancing expansion in the syringe itself by the expansion of the rods (SS in Fig. 1).

By choosing materials having suitable coefficients of expansion, the attempt was made to produce a syringe-pipette that would deliver the same volume of fluid at any reasonable working tem-

* Small air bubbles which remain in the syringe do not affect the accuracy of the delivery unless they are subjected to variable degrees of compression.

perature. The results were thoroughly satisfactory as judged by a series of calibrations covering the temperature range 10° to 26°. The results are tabulated below :

Syringe number.	Temp. of air, syringe, and water.	Mean wt. of H_2O delivered, g.	Vol. calculated from density of H_2O (referred to 4·0°).
1	10·0°	1·50381	1·50422
	19·5	1·50193	1·50443
	25·5	1·49997	1·50457
2	20·0	1·52310	1·52580
	26·0	1·52083	1·52570
3	11·0	1·33014	1·33063
	20·0	1·32834	1·33070

Mention has already been made of the means whereby the amount to be delivered may be varied at will. With the stop screws we have been using (18 threads per cm.), each revolution of the screw corresponds to about 0·0160 c.c. On the smooth shank of each screw is a longitudinal line; the position of this line is referred to graduations on the top plate. It is a matter of but a few minutes to change the delivery to any desired amount within the range of the instrument. In general, we find that four fillings and deliveries are made with the syringe-pipette in the same time that one is made with other pipettes.

Since the syringe-pipette is designed primarily for analytical purposes, it seems pertinent to mention briefly some of the reasons why it is peculiarly adapted to the requirements of precise analysis. Most volumetric analyses are titrimetric in nature. In general, the accuracy of a titrimetric determination ultimately depends on the concentration in which the titration is made, so that the smaller the volume at the end of the titration the greater is the theoretically attainable accuracy, a fact which has been discussed by Bjerrum (" Die Theorie des alkalimetrischen und azidimetrischen Titrierungen," Stuttgart, 1914) and Rehberg (*Biochem. J.*, 1925, **19**, 270). Within the range of applicability the same will be true of colorimetric or nephelometric determinations. In other words, it is more advantageous to measure small volumes with high accuracy than it is to measure larger volumes with the same relative accuracy. The fact that the amount of material available for analysis is seldom indefinitely large is another, and frequently compelling, reason for using small volumes. Everything indicates the desirability of using concentrated reagents in small amounts; these are delivered by the syringe-pipette, as we have seen, with far greater accuracy than otherwise possible.

From the very reason of the higher concentrations used, it follows that the quantities employed must be more delicately adjusted to one another. This is particularly true of so-called " back-titrations " where an excess of a reagent is titrated. It is here that the adjustable volume of the syringe is almost indispensable.

Summary.

A syringe-pipette is described which extends considerably the range of useful accuracy of volumetric procedures and delivers with great rapidity volumes of the order of 1—2 c.c. with a precision of 0·1 c.mm. The volume to be delivered is adjustable according to the needs of the operator.

Changes in temperature, within ordinary working limits, have no significant effect on the volume delivered by the syringe-pipette, the independence of the temperature being achieved by counteracting expansion with another expanding system.

The application of the syringe-pipette to chemical analysis is briefly discussed.

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