CVII. AN IMPROVED DESIGN OF THE VAN SLYKE APPARATUS FOR THE ESTIMATION OF AMINO-NITROGEN.

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AN extended use of the apparatus described by Van Slyke [1912, 1914, 1915] for the quantitative determination of amino-nitrogen has revealed several drawbacks.

There are three points in the glass system at which pieces of thick-walled glass capillary tubing are joined by rubber tubing. The undesirability of rubber connections in systems in which gas volumes are measured needs no emphasis. In the case of the Van Slyke apparatus the abolition of the rubber connections is the more desirable in that shaking of the deaminising chamber and Hempel pipette subjects the rubber to periodic stretchings which must diminish its impermeability to gases. Where deamination takes 1 hour, as it does in the case of lysine, histidine, etc. [Plimmer, 1924], this disadvantage may be serious. It was found necessary always to apply glycerol to the surfaces of contact of glass and rubber and tightly to wire the rubber to the glass.

If thick-walled rubber tubing is used in order to lessen the danger of loss of gas at the joints, shaking subjects the thick-walled glass capillary tubing to strain, on account of the elasticity of the rubber. In use, breaks were frequently found to occur.

These considerations prompted the design of a new form of the apparatus in which the disadvantages referred to were eliminated.

The gas system is made in one piece and mounted without strains on a stout, upright board, having apertures cut in it where necessary. Fig. 1 shows a front view of the apparatus. *A* represents an end elevation of the deaminising bulb which is of the original Van Slyke design. The deaminising bulb is clipped to a wooden block, which is screwed to the board and is not indicated in the figure. The thick-walled capillary tubing as well as the two-way tap *B* are of larger size than usual in order to impart greater strength to the apparatus.

The gas burette is of 10 cc. capacity, graduated in its upper part in 0.01 cc., the bore being as large as conveniently possible. The bulb at its lower end holds 40 cc. A bulb of this size was found necessary when hexone bases were analysed,

since at the end of the hour necessary for deamination considerably more than 20 cc. of gases have been evolved.

Fig. 1.

The levelling manometer *C* provides greater ease and accuracy in adjusting gas volumes to atmospheric pressure. It is bent over at right angles at the top and fitted with a tap *D*, shown in Fig. 2, so that the manometer can be closed when the manipulations necessitate it. Its diameter should be large enough for the capillary rise of water to be negligible.

The absorption pipette has been slightly modified from the original, the spherical bulb being of 100 cc. capacity, while the cylindrical vessel holds 60 cc.

A thermometer *E* and waste pipe *F* are also fastened to the board, the latter communicating by rubber connections with the three outlets of the deaminising bulb.

The upright board is fastened by strong hinges at the bottom to a base-board and can be oscillated about the hinges by means of a crank attached to the board by the brass coupling *G*. The crank is actuated by the wheel *H*

driven by an electric motor *J*, a considerable variation in the speed of shaking being afforded by a suitable rheostat. The whole apparatus is firmly screwed to the bench.

Fig. 2.

The levelling reservoir is placed in a cradle which can be slowly raised or lowered by means of an endless screw.

With this apparatus it was found possible to estimate amino-nitrogen to 0.01 mg. A standard solution of twice crystallised copper aminoacetate was prepared and the quantity of amino-nitrogen in 2 cc. determined, the solution being run in from a calibrated pipette and washed in with 2 cc. water.

Barometer mm. mercury	Temp.	cc. nitrogen evolved	mg. amino- nitrogen
765	18.8°	1.98	1.14 (1)
765	19.0	1.99	1.14 (6)
767	20.0	1.96	1.12 (7)
767	21.0	2.00	1.14 (4)

Theoretical value 1.13 mg.

In 1915, Van Slyke described a modification of his original apparatus in which, it was claimed, volumes of nitrogen could be measured to 0.001 cc. by means of a burette graduated in 0.01 cc. with the graduations about 1 mm. apart.

It seems doubtful whether this degree of accuracy can generally be attained without special precautions to ensure the constancy of the temperature of the gas burette and also to ensure that there is no temperature lag between the latter and the thermometer. A temperature variation of 0.5° will make an appreciable difference in the third decimal place of milligrams of nitrogen.

Further, it seems inadvisable to use a gas burette of so narrow a bore as that specified by Van Slyke, since in such a tube the capillary rise of water will be appreciable. Hence the gaseous volumes will be read under a slightly different pressure from that recorded by the barometer.

During the manipulations it is almost impossible to avoid contamination of the water in the upper part of the burette with acetic acid, sodium nitrite, potassium permanganate and sodium hydroxide, and it is certain that such capillary active substances will appreciably affect the surface tension of the liquid over which the gas is measured and hence change the capillary rise. The pressure of water vapour in the burette will also be changed by the presence of these impurities.

Though the magnitude of these effects is not accurately known, consideration of them makes it evident that, until they have been shown to be negligible, volumes of nitrogen under these conditions can only be given with certainty to 0.01 cc.

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