NEW APPARATUS

A TWO-STAGE MICRO-EVAPORATOR

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Two devices for concentrating solutions are described. The first reduces a volume of 1 ml. to one of 0·05 ml. and the second a volume of 0·05 ml. to a few microlitres.

Recent developments in microchemical techniques make possible the identification of sub-microgram quantities of various compounds including alkaloids[1], sugars[2,3], steroids[4] and inorganic radicals[5]. These techniques need a moderately concentrated solution. Therefore to obtain high sensitivity, a very small volume (in general 0·1 μl.[6]) has to be employed. This may give rise to difficulties. For example, if a test will detect 0·01 μg. of a compound dissolved in one micro-drop (0·1 μl.), a solution containing 1·0 μg. in one ml. will have to be concentrated 100 times (i.e., to a volume of 10 μl.) to give a positive result. To effect such concentration is quite impracticable by ordinary means, and is even beyond the limit of the device described by Tryhorn and Curry[7]. To resolve this problem the two pieces of apparatus described below have been developed.

It is assumed that there is no difficulty in reducing a volume to 1 ml. by ordinary methods.

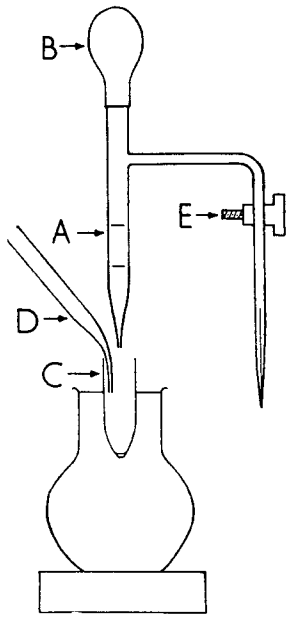

Fig. 1. First-stage evaporator.

Description of Apparatus

The first-stage evaporator will reduce the volume from 1 to 0·05 ml. The solution to be evaporated is drawn up into the tube A by means of the rubber bulb B (Fig. 1). It is allowed to drip slowly into the small conical tube C, which is maintained at a temperature of about 65° by means of a miniature water bath made from a small, flat-bottomed, bolt-head flask standing on a Simmerstat-controlled hotplate. Evaporation is assisted by means of a jet of air or nitrogen from the tube D. The optimum rate of dripping, which is controlled by the adjustable air-leak E, is such that one drop is evaporated to dryness before the arrival of the next. This has the effect of concentrating the residue on a small area at the bottom of the tube. It is usually more convenient to take the solution to complete dryness, and to redissolve in a volume of 0·05 ml. The device works best

with a solvent such as chloroform or methanol which boils at about 65°, but may also be used with less volatile liquids such as ethanol or water.

In many cases this 20-fold concentration is sufficient, but if tests carried out with micro-drops of the final solution are still negative, further concentration may be effected with the second-stage evaporator (Fig. 2).

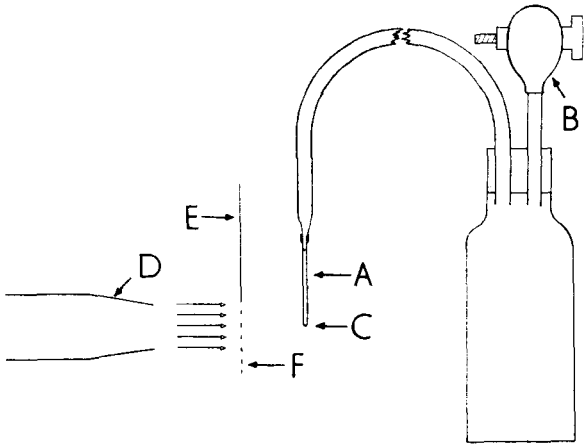

FIG. 2. Second-stage evaporator.

The solution (0·05 ml.) is drawn up into the melting point tube A by manipulation of the screw-controlled rubber bulb B, which is then adjusted so that there is a small positive meniscus at C. This is subjected to a stream of hot air from the blower D. A metal shield E prevents overheating of the liquid in A, while a gauze F moderates the air-flow to prevent the drop at C being blown away. The position of E and F respectively may be varied in relation to A. The bulb B is adjusted continuously to maintain the pendant drop at C. This drop should be kept small to avoid loss of material on the outside of the tube. When the column of liquid in A is reduced to less than 0·25 cm. in length the air blower may be switched off. Micro-drops of the concentrated solution in A may be taken for use with colour or crystal tests, or the whole of the remaining liquid may be transferred to a suitable surface and used to carry out a single reaction.

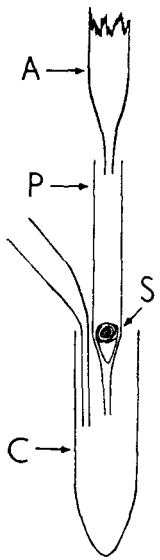

Fig. 3. Elution from paper chromatograms.

APPLICATIONS

By this method it has been found possible to demonstrate the presence of 1 part of strychnine in 10^6 parts of ethanol[1], 1 part of progesterone in 10^6 parts of methanol[4] and one part of glucose in 10^4 parts of water[2,3]; one millilitre of the solution having been used in each instance.

The first stage evaporator is especially useful in the elution of material from a paper chromatogram. For this purpose a Pasteur pipette P is inserted between the dropper A and the conical tube C (Fig. 3). The chromatogram spot is cut out and, after appropriate treatment, is folded up and forced into the neck of the pipette (S). A suitable solvent is drawn into A and allowed to drop slowly via P into C, eluting the substance from S as it passes. As before, the eluate is deposited at the bottom of C and may be redissolved in a minimal volume of liquid for subsequent examination.

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References

1. Clarke and Williams, *J. Pharm. Pharmacol.*, 1955, **7**, 255.
2. Clarke, *J. Physiol.*, 1957, **135**, 28P.
3. Clarke, *Biochem. J.*, 1959, **72**, 13P.
4. Clarke and Taylor, *J. Physiol.*, 1960, **151**, 14P.
5. Clarke, *Analyst*, 1959, **84**, 662.
6. Hawkins, *Brit. J. appl. Phys.*, 1959, **10**, 99.
7. Tryhorn and Curry, *Nature*, Lond., 1956, **178**, 1180.