IMMOBILIZED ENZYME PIPETTE - 'IMPETTE'

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A new device that uses immobilized enzymes and is called an Immobilized Enzyme Pipette or 'Impette' is made by attaching disposable pipette tips made of polymeric nylon tubes containing enzymes attached covalently to their inner surface to the stem of an automatic, adjustable-volume pipette holder. This paper describes the preparation and application of this new device in research laboratories and as a routine analytical device in clinics. Other possible applications are also discussed.

MATERIALS AND METHODS

Nylon tubing (i.d. 1 mm) was supplied by Portex Ltd., Hythe, Kent, U.K., Urease type III (28 Units/mg) was obtained from Sigma Chemical Company, St. Louis, USA. All other chemicals were purchased from Aldrich Chemical Company, Milwaukee, USA.

Disposable pipette tips (Impette tips) containing enzyme were made by coupling enzyme to nylon tubing by the method of Sundaram et al (1,2).

CONSTRUCTION OF AN IMPETTE

An Impette may be constructed by attaching the disposable pipette tips containing the immobilized enzyme to the stem of an automatic, adjustable-volume pipette as seen in Figure 1.

The Impette tips were stored soaking in a buffer of choice at 40°C when not in use.
RESULTS AND DISCUSSION

CALIBRATION AND PRINCIPLE OF OPERATION

A kinetic or an end-point method (equilibrium method) may be used in the determination of specific substrates in unknown solutions using an Impette. A standard solution of 1-50 mM urea made up in pH 7 phosphate buffer containing 1 mM EDTA was sucked into an Urease-Impette whose volume was adjusted to 0.25 ml, allowed to react for exactly 5 minutes and then expelled. The amount of ammonia formed during the reaction was estimated as described in Sundaram et al (1). A standard curve was prepared using the same method with various substrate concentrations.

In routine determination, unknown samples were treated in exactly the same manner as the standards and the A630nm values obtained from ammonia determinations were read off against the standard curve to arrive at the concentration of urea in the sample. This kinetic method for the determination of urea permits the assay to be completed in 5 minutes during which only a portion of the substrate is converted to product. Thus, it is important to time the reaction exactly with a stop-watch.