IN THE LABORATORY

A Simple Micro-Beaker for Use With the Beckman pH Meter (Model G)

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It is often necessary in certain microbiological procedures to determine the pH of a minute quantity of a fluid which may not possess a sufficient buffer capacity to permit dilution to the minimal quantity of approximately 2 ml, as required in the ordinary specimen cup (5-ml beaker). Other procedures, occasionally requiring subsequent analyses on the same minute amount of specimen, may not permit of dilution despite the inherent buffer capacity. The use of the device to be described permits one to make more frequent tests on such substances as tears, sweat, salivary aspirate, and other normal as well as pathological fluids ordinarily obtainable

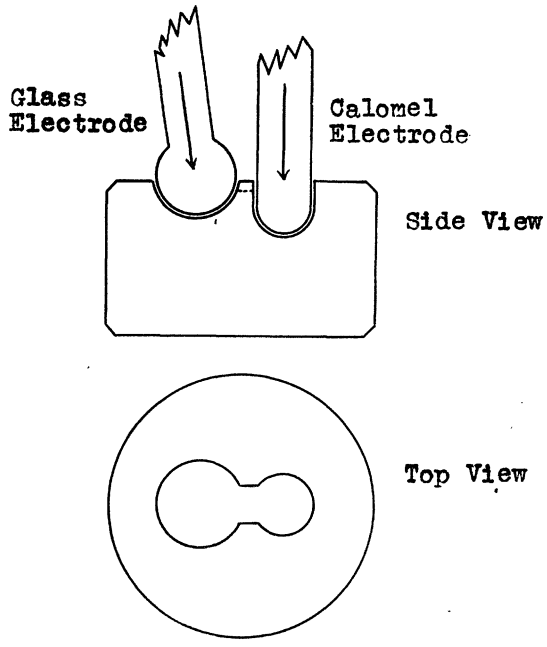

FIG. 1

in only limited quantities. Certain clinical and pediatric specimens of but a single drop in quantity may be accurately determined in only a fraction of the time that is usually required for a microcolorimetric test.

The micro-beaker shown in Fig. 1 was cut from a $\frac{3}{4}$" cast Lucite rod. Plexiglas, a virtually identical methyl methacrylate product, may also be used for this purpose. A length of $\frac{1}{2}$" is cut from the rod and affords a most serviceable height. A $\frac{3}{16}$" and a $\frac{1}{4}$" round surgical bur were used to cut the circular depressions to the depths shown. The channel between the depressions is cut with a $\frac{1}{8}$" round bur (surgical or dental). The channel, of course, establishes the necessary bridge between the electrodes at the expense of a negligible amount of the specimen. The depressions and channel are polished with a wisp of cotton wound around the burs employed, using extra fine pumice and water. A thin paste made of SnO_2 in water produces a lustrous and smooth finish. Various hand rotor devices or the dental engine serve admirably in performing these operations. In order to avoid depolymerizing the surface of the plastic the cutting procedures should not be performed too rapidly, and even greater discretion should be employed in avoiding the generation of heat in polishing. The entire device may be smoothed and polished with the same agents, using a polishing wheel of medium size. This design is applicable to the Beckman "270" calomel electrode and the "290" glass electrode, which are most commonly employed with the instrument.

The total volume of the depressions and channel should not exceed 0.2 ml. However, 0.1 ml of the specimen may be expediently employed due to the combined displacing volumes of the two electrodes. A simple procedure is to place a drop, or about 0.05 ml, in each depression, place the micro-beaker in the specimen-cup support (beaker holder), loosen both electrode set screws, and carefully elevate to position. The calomel electrode should follow the path of straight-line insertion, the glass electrode being converged toward it. The comparatively soft and resilient surface of the plastic greatly minimizes the danger of breaking or scratching the electrodes. The bridge will automatically be formed if the fluid should not be inordinately viscous. Care should be taken with viscous solutions, however, for if any air is trapped in the smaller depression, connection with the porous fiber of the immersion tip of the calomel electrode cannot be established. The diameter of the micro-beaker being less than that of the cup support (beaker holder) ensures simple flexibility in adjustment. The use of this device does not require the removal of the regular glass electrode, or the 5-ml beaker holder, and one worker may immediately follow another using the micro-beaker or conventional cup without inconvenience. The temperature variable is no more critical with 0.1 ml of a specimen than with a larger amount.

The plastic is entirely resistant to acid or alkali within the usual pH range (2–10). It is readily attacked by the organic solvents and crazes rapidly by short subjection to 70% alcohol. The micro-beaker is best cleansed with a soft brush or towel, using warm water, soap, or any of the detergents. It may be disinfected, if necessary, by immersion in a 1:1,000 solution of bichloride of mercury for 10 min or a 1:1,000 solution of benzalkonium

chloride (U.S.P.) for 20 min. Because of the aforementioned effect that alcohol has upon the plastic, tinctures must necessarily be avoided. Boiling of the device causes cloudy depolymerization and is therefore inadvisable. The ½″ height of the device permits the intact removal and subsequent replacement of the specimen cup without replenishment of the distilled water or buffer solution in which the electrodes are ordinarily maintained.

When testing minute quantities by this method it is particularly desirable that the electrodes and microbeaker be scrupulously clean and adequately rinsed. The electrodes are ideally rinsed three times and blotted with a fresh sheet of cleansing tissue. This procedure is especially recommended if the specimen should be poorly buffered. For a very critical test a contact discrepancy in readings may be avoided by employing the device with the preferred buffer solution when making the asymmetry potential corrections. It is also preferable to use a buffer solution of approximately the same pH value as the solution being tested. The plastic is comparable to soft glass as a nonconductor, possesses less porosity, and is virtually unbreakable.

A Rapid Method for Preparing DDT in the Laboratory[1]

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DDT is an abbreviation of the chemical term, dichlorodiphenyl-trichloroethane, $(C_6H_4Cl)_2CHCCl_3$. This outstanding new insecticide has become, during the last 5 years, the subject for numerous investigational studies in the entomological and chemical fields. The commercial technical-grade DDT is a mixture of several isomers and impurities (*3*). It consists essentially of 70–75% of *p,p′*-DDT, 1-trichloro-2,2-*bis* (*p*-chlorophenyl) ethane. The *p,p′*-DDT isomer is mainly responsible for the insecticidal properties of the compound. When pure, it has a melting point of 108°–109° C.

Commercially, DDT is now prepared by the Baeyer condensation method originally employed by Zeidler (*5*), who was the first to report the synthesis of this compound. The process involves the use of chloral, fuming sulfuric acid, expensive and more or less complicated apparatus (*4*). Both chloral and fuming sulfuric acid are unpleasant and hazardous to handle, especially by inexperienced workers and students. Recently a laboratory method was suggested by Darling (*2*) in which chloral hydrate is substituted for chloral but requires the addition of oleum.

Research work with DDT, as well as teaching insecticides, often requires the synthesis of small quantities of pure DDT in the laboratory. Not all laboratories are equipped with suitable apparatus to handle hazardous reagents. The writer, being confronted with such a problem, has worked out a short and simple method for preparing small quantities of DDT without the use of either chloral or fuming sulfuric acid. The entire process can be carried out within one laboratory period and has been successfully used during the last two years by the writer and graduate students taking courses in insecticides.

The method is based on the theoretical reaction of 1 gm mole of chloral hydrate and 2 gm moles of chlorobenzene (sp.gr., 1.107) in the presence of about 4–5 times their combined volume of concentrated sulfuric acid.

When only small quantities of DDT are desired, correspondingly smaller proportions of ingredients, based on 0.1 gm mole of chloral hydrate, such as 17 gm of chloral hydrate and 23 ml of chlorobenzene, are convenient to use.

The procedure is as follows:

Place 17 gm of chloral hydrate crystals and 23 ml of chlorobenzene in a glass-stoppered, 500-cc Pyrex reagent flask and keep in electric oven at 60°–70° C for about 20–30 min with occasional shaking, or until all the crystals have dissolved. Cool to room temperature and slowly add about 180 ml of concentrated H_2SO_4. Stopper and shake vigorously until precipitation starts. This operation usually requires about 1 hr. Let stand for about 15 min with frequent shaking or until precipitation is complete. The upper solid layer contains the crude DDT.

Pour the mixture into a glass jar containing about 1 gal of cold tap water and allow to stand for 15 min, or until the solids have settled. Filter through three layers of cheesecloth and wash several times with tap water. Transfer residue from cheesecloth into a wide-mouth bottle or a small glass jar, add about 50 ml of either 2% Na_2CO_3 or 4% $NaHCO_3$ solution, and shake for 5–10 min. This will neutralize the acidity.

Filter through a small, dry, Buchner funnel and wash several times with distilled water, or until the filtrate is neutral to litmus. Continue air suction for a few minutes or until no more water drains out. Transfer the residue to a small porcelain mortar, add about 100 ml of ethanol, and triturate with a pestle for 5–10 min. Filter through a dry Buchner funnel, rinsing twice with 25 ml of ethanol. Continue suction until no more alcohol drains out.

Dry the residue at 70°–75° C in an electric oven for 2 hrs, or until all the alcohol has volatilized. Cool to room temperature and weigh. An average yield of about 16 gm of practically pure DDT is obtained.

Samples from 6 batches prepared during the last two years were analyzed for *p,p′*-DDT by the chemical method developed by Cristol, Hayes, and Haller (*1*). The results have shown variations of from 94.5 to 98.35% *p,p′*-DDT, giving an average of 96.82%. The melting point ranged from 106° to 108° C.

The laboratory-prepared DDT was tested on third-instar larvae of *Aëdes aegypti* in comparison with a commercial sample of DDT (technical grade). The tests were made in beakers, each containing 200 ml of distilled water and about 50 larvae. One ml of ethanol containing various concentrations of the toxicants was dispersed in each beaker. Each concentration was run in triplicate, giving a minimum of 150 larvae per test.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Entomology.