Communications

Simple Manual Pipette for Toxic Reagents

Because of the many varied chemical reagent solutions in use in our laboratory for biochemical analyses, the need arose for a safe method of pipetting toxic solutions to avoid getting any dangerous or toxic solutions in the operator's mouth, either by carelessness, by fumes, by overpipetting, or by air traps.

A simple automatic pipette was therefore devised and constructed to handle small volumes of toxic reagents (*1*). It has been used in this laboratory to pipette silver nitrate reagent, sodium hydroxide solutions, concentrated ammoniacal solutions, sodium cyanide reagent, sulfuric, nitric, and trichloracetic acid solutions, phenol reagent, and molybdate-sulfuric acid reagent.

The component parts are a standard bottle with a standard taper mouth, a standard taper ground joint, and a standard pipette, of Pyrex glass, with the ring seal and the side-arm tube fabricated by hand (*2*). The complete assembly is interchangeable so that a 1-liter bottle pipette can be cleaned and used in another 1-liter bottle. We have used both 5- and 10-ml pipettes and 500- and 1000-ml bottles for various reagent solutions, and they have exhibited excellent strength in usage.

The automatic pipette reagent bottle (Fig. 1) consists of a 1000-ml Pyrex reagent bottle *A* with S.T. opening fitted with a 29/42 S.T. Pyrex ground joint *D*. The ring seal *C* is made by sealing a 5- or 10-ml red-line Pyrex measuring pipette *B* to the S.T. ground joint well above the top graduation of the pipette so that the accuracy of the pipette is not altered. The rubber pressure bulb *G* is fitted to an 8-mm tube that is sealed to the ground joint *H*. The thumb safety air-pressure release *F* is a small 8-mm opening, slightly flared and fire-polished. The S.T. joint fitting *E* for the bottle is lubricated with silicone stopcock grease (*3*) to facilitate both ease of removal of the entire assembly and a clear view of the graduations when making measurements. A small washer *J* of tygon tubing is fitted below the top of the pipette, and when not in use, a Pyrex Wasserman tube *K*, cut in half and packed with glass wool in the sealed end, acts as a closure for the mouth of the pipette to minimize contamination or evaporation of the reagent or solution in the bottle. In use, manual pressure is exerted on the rubber bulb, with the thumb sealing the safety opening *F*, forcing the solution in the bottle up into the pipette. When the solution is above the zero mark but below the mouth of the pipette, the safety opening is released, allowing equalization of air pressure, and the forefinger is immediately applied to the opening of the pipette. The pipette assembly is then removed from the bottle and the excess solution allowed to run back to the zero mark. The solution in the pipette, with the forefinger as control, is then dispensed as usual in any amount up to 10 ml.

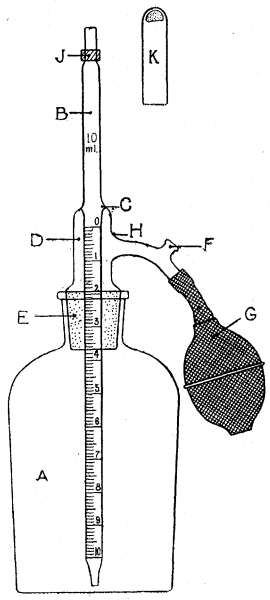

Fig. 1. Pipette for toxic reagents.

The actual filling and zero adjustment of a 10-ml sample of solution in this apparatus takes less than 15 sec and it has proved satisfactory to the personnel who have been using it. More than six of these assemblies have been in constant daily use for a 4-mo period without breakage or freezing of the closure.

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Notes

1. This pipette has been found useful during technical procedures carried out in The Johns Hopkins Hospital on a project supported by a contract between the U.S. Atomic Energy Commission and The Johns Hopkins University with Dr. John Eager Howard as Research Contract Director.
2. Made to specifications by T. Elmo Maiolatesi, Baltimore, Md.
3. Dow-Corning Silicone Stopcock Grease, which has proved impervious to most of the reagents we have used with the pipette assembly.

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Constant Current or Constant Voltage?

It is sometimes stated that for paper electrophoresis one needs *constant current* not *constant voltage*. The very active development of paper electrophoresis now going on makes it desirable to consider the basis of this statement. The following analysis shows that it is sometimes true and sometimes not.

The *desideratum* in electrophoresis is a known, constant, and reproducible electric field for which the field strength *E* can be measured in volts per centimeter.

The direct calculation of E involves dividing the voltage difference between the ends of the migration path by the path length. In the usual type of free-solution electrophoresis cell, the direct measurement of voltages in the migration path is inconvenient and one may then resort to an indirect method based on the equation $E = I/KA$, which assumes that the conductivity K is constant and the cross-sectional area A is uniform. With these conditions satisfied, a constant-current supply insures the required electric field.

The situation is not the same in paper electrophoresis. Here we must distinguish between (i) apparatus in which the paper is exposed to an atmosphere and (ii) apparatus in which it is pressed between two solid surfaces.

When the paper is exposed to an atmosphere, whether saturated or not, evaporation from the paper occurs because of heat generated by the electric current. The quantity of heat H is calculated for a constant-current supply from the equation H (cal/min) $= 14.3\ RI^2$, and for a constant voltage supply from the equation H (cal/min) $= 14.3\ V^2/R$. Calculations based on a paper strip carrying 7 ma at 500 v indicate that as much as 0.1 ml of water may be evaporated per minute. A sequence of events is thereby initiated (concentration of buffer, influx of water from electrode compartments, and decreased resistance), which, with a constant-voltage supply, results in increased current density, increased heat production, and still more evaporation. At higher voltages, the cycle may proceed to the point of igniting the paper. This occurred in one case while operating at room temperature with 1000 v across a 30-cm strip of paper using 0.1M barbital buffer.

On the other hand, a constant-current supply, while it does not prevent the change in conductivity, does control the heat production, but at the cost of a constantly decreasing applied voltage as the resistance decreases. In this case, therefore, a choice must be made between constant-voltage supply with the danger of overheating and constant-current supply with the disadvantage of varying migration rate.

When the paper is compressed between two solid surfaces—for example, glass plates—evaporation is completely prevented and the temperature rise is controlled by the temperature of the contact surfaces. If no other disturbing factors are present, either constant-current or constant-voltage supply can be used. However, it is found experimentally that even with constant pressure the conductance of the paper-buffer system increases during the run, although not as much as in the exposed strip, apparently because of electroosmotic influx of buffer. The constant-voltage supply is therefore preferred, since it has the advantage of producing constant field strength. The field strength is calculated by dividing applied voltage by paper length (measured between buffer surfaces).

The subject is treated more fully by the writer in a forthcoming monograph, "Paper and zone electrophoresis manual" (E-C Apparatus Co., New York, 1954).

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Glass-Fiber Paper Impregnated with Silicic Acid as a New Chromatographic Tool

In attempting to separate saturated mono-, di-, and triglycerides by paper chromatography, it was found difficult to locate the spots, because the paper is destroyed by the drastic tests necessary to locate these compounds. To overcome this difficulty, a fine glass-fiber filter paper was obtained, which, when impregnated with silicic acid, was found to have chromatographic properties similar to that of silicic acid columns.

D. L. Fillerup and J. F. Mead [*Proc. Soc. Exptl. Biol. Med.* 83, 574 (1953)] were able to separate a mixture of triglycerides, fatty acids, cholesterol, and cholesterol esters on a silicic acid column by using increasing amounts of ethyl ether in petroleum ether as eluting solvents. It was found in this laboratory that mono-, di-, and tripalmitin, cholesterol, and cholesterol acetate can be separated on glass paper impregnated with silicic acid using a developing solvent consisting of a 2-percent ethyl ether in isooctane. Typical R_f values obtained were as follows: 1-monopalmitin, 0.05; dipalmitin, 0.27; cholesterol, 0.41; tripalmitin, 0.79; cholesterol acetate, 1.0.

The location of the sterol spots was accomplished by spraying one side of the chromatogram with the Liebermann-Burchard reagent followed by heating over an electric pot plate with exposed heating element. Cholesterol and cholesterol acetate appeared as bright pinkish-red spots on a white background. All areas containing carbon compounds were located by spraying the reverse side of the chromatogram with a dichromate-sulfuric acid-water solution followed by heating over the hot plate. All areas containing carbon compounds appeared as light to dark grayish-black spots on a yellowish-orange background. The color of the spot depended in large measure upon the amount of carbon present.

This technique is being adapted to the separation of other groups of compounds. The details of the procedure will be published later.

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