Adaptation of the Silicomolybdic Acid Method for the Estimation of Morphine to the Photoelectric Colorimeter

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In 1937 Snell and Snell (*2*) published a method for the quantitative determination of morphine in tissues and biological fluids, based upon the fact that morphine reduces silicomolybdic acid in alkaline solution to a blue product suitable for colorimetric analysis. These authors found determinations by this method to be accurate to about 2 per cent for 2–7 mg. of morphine. One of its advantages is that silicomolybdic acid is not reduced by any of the constituents present in a trichloracetic acid extract of tissue slices after incubation (*1*).

We have modified and adapted this method for use on a semimicro scale with the Evelyn photoelectric colorimeter. A strict proportionality is obtained up to 0.7 mg. of morphine, with only slight deviation from a linear relationship above this amount.

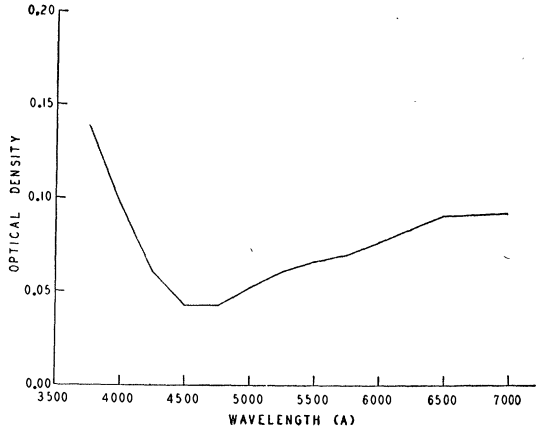

FIG. 1

A standard curve is obtained by introducing various amounts of morphine sulfate (0.1–1 mg.), in 5 cc. of distilled water, into 25-cc. volumetric flasks. This can be accomplished by adding various amounts (0.5–5 cc.) of 0.02 per cent morphine sulfate· 5 H_2O to the flasks, followed by that amount of distilled water necessary to make a total of 5 cc. Next, 4 cc. of silicomolybdic acid reagent, prepared according to the method described by Snell and Snell, is added to each flask, followed by 0.5 cc. of 5 per cent trichloracetic acid. Finally, 10 cc. of 5 per cent ammonium hydroxide is added. A reagent blank in which morphine is omitted is also prepared. The volume is diluted to 25 cc. with distilled water, shaken vigorously, and allowed to stand at room temperature for 25 minutes. It is then transferred to colorimeter tubes and read on the Evelyn photoelectric colorimeter, using the 10-cc. aperture and a No. 660 filter. The reagent blank is used to obtain a center setting. Spectrophotometric study showed a plateau of maximum absorption, in the visual range, between 6,700 and 7,000 A. (Fig. 1), with a possible peak in the ultraviolet which could not be investigated with existing facilities. Filter No. 660 has transmission limits at 635–720 mμ. L (photometric density) is plotted against the amount of morphine sulfate present in milligrams (Fig. 2, A).

Recoveries from tissues incubated with morphine were also carried out, using more than 0.5 cc. of 5 per cent trichloracetic acid (per 25 cc. total volume) to stop enzymatic activity; recoveries ranged from 93 to 110 per cent. However, 0.5 cc. of this reagent seems to be optimal for good linearity above 0.7 mg.; it is therefore suggested that, in recoveries of morphine from tissues, enzymatic activity be stopped by some other protein precipitant, rather than by adding more than the optimal amount of trichloracetic acid.

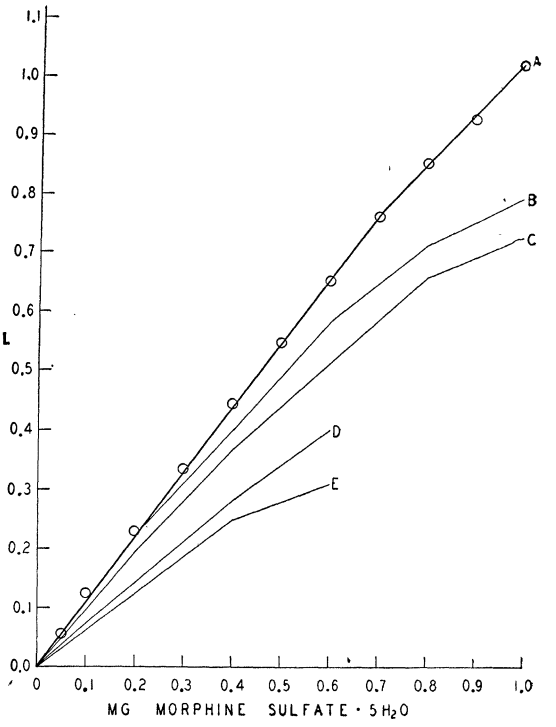

FIG. 2

Some attempts were made to determine the mechanism of the specific action of trichloracetic acid in increasing the sensitivity of the method and the range of the determination. It was found that the pH of the final solutions, prepared as outlined in the method and containing 0.5 cc. of 5 per cent trichloracetic acid, was 9.94–9.98. This final pH was reproduced by substituting, for the trichloracetic acid in the solutions being deter-

mined, sufficient amounts of 5 per cent acetic acid (Fig. 2, E), 0.1 N hydrochloric acid (D), 5 per cent monochloracetic acid (C), and 5 per cent dichloracetic acid (B), respectively. The ability of these acids to increase the sensitivity and range of this method *decreased* in the following order: trichloracetic, dichloracetic, monochloracetic, hydrochloric, and acetic acid. This would suggest that the specificity of trichloracetic acid in affecting this method is a function of the number of chlorine atoms attached to the carbon adjacent to the carboxyl group and not a matter of pH or the acetate radical itself.

References

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2. SNELL, F. D., and SNELL, C. T. *Colorimetric methods of analysis, II.* New York: D. Van Nostrand, 1937. P. 510.

Use of Phenol Formaldehyde and Vinyl Resins in Sealing Liquid Mounting Media on Microscope Slides

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A persistent difficulty in the preparation and preservation of certain types of plant materials on microscope slides is the problem of permanently sealing liquid mounting media. Glycerin, lactic acid, lactophenol, and certain other mounting fluids of diverse composition are exceedingly difficult to inure against changes in atmospheric humidity, temperature, and slow chemical reaction with the sealing agent. The dependence upon liquid media for mounting certain plant tissues, although unfortunate, is necessitated by requisite indices of refraction and other physical and chemical properties of diverse mounting fluids. It therefore becomes necessary to employ a sealing compound which gives a reasonable promise of permanence if extensive collections of such plant materials as pollen grains, leaf cuticles, fungus mycelia, and other whole mounts are to be maintained for permanent reference.

Recently, in the preparation of reference collections of root and leaf epidermal tissues for use in the identification of plant fragments in peat and lignitic coals, a need was realized for permanent sealing of glycerin and lactic acid preparations of such tissues. Consideration of the physical and chemical behavior of various synthetic resins and plastics indicated the possibility that phenol formaldehyde and vinyl resins might provide the necessary physical properties and chemical resistance. Preliminary tests showed a p-phenyl phenol formaldehyde resin and a vinyl acetate resin to be very promising for prolonged sealing of noncorrosive and certain corrosive media. These resins, when applied copiously with a brush or pipette to clean glass surfaces, form an effective seal which is not altered by prolonged storage at normal temperatures. In the case of the phenolic resin an accelerated aging test involving 8 months storage at 50° C. with frequent intermittent changes to room temperature has resulted in perfect sealing of lactic acid and glycerin preparations. The vinyl resins have not been subjected to as rigorous tests, but appear to be equally effective.

The phenolic resin showing the most desirable properties for a sealing agent is a p-phenyl phenol formaldehyde compound containing tung oil and a metallic soap as oxidizing agent. The resin is prepared and sold as a commercial varnish and has the syrupy consistency of a thick oil. The setting of the liquid to a solid is complex chemically, involving solvent evaporation, oxidation of the tung oil, formation of colloidal systems, and polymerization of the resin. The dried and polymerized resin is exceedingly hard, yet slightly elastic, shows extreme adhesiveness to glass, and is extraordinarily inert chemically. When cured by drying and especially by heating to about 50° C., the resin is completely inert to ordinary organic solvents such as alcohols, hydrocarbon solvents, and halogenated hydrocarbons. Resistance to mineral acids was tested by ringing lignin residues of wood sections prepared with 72 per cent sulfuric acid. The resin shows effective sealing of the strong acid after 3 months storage at 40° C. A slight carbonization at the edges of the cover glass occurred shortly after sealing, presumably by action on the tung oil. In the case of organic acid media such as lactic acid and lactophenol, clouding of the resin may occur near the interface with the mounting fluid. This cloudiness does not appear to progress after polymerization of the resin at 50° C., and no tendency has been observed for the mounting fluid to become cloudy by emulsification of the sealing agent, as is frequently the case with compounds such as Noyers cement after prolonged storage.

For best results the p-phenyl phenol formaldehyde resins should be kept in a large stock container of quart capacity or more and drawn off only when needed. Small portions of the liquid resin, when kept in dropping bottles or similar containers, set to a gel within a day or two and are useless after jelling since the material becomes insoluble in organic solvents. When still liquid, the resin may be thinned with neutral xylene or ethyl acetate. The tendency to gel after exposure to air can be obviated by proper handling.

Slides, after ringing with the resin, may be dried for 8-24 hours and placed on a warming table or in an incubator to complete the polymerization of the resin. Some change in the color of the resin accompanies polymerization, resulting in an amber yellow. The resin shows no wrinkling by shrinkage after slow drying and polymerization on the glass. Clean glass surfaces are essential for perfect sealing, although excess glycerin or other liquid at the edges of the cover glasses may be effectively sealed if it is not sufficiently copious to form a film between resin and glass.

Phenolic resins of the type described here are produced by the Bakelite Corporation, New York City. The commercial product used in the writer's laboratory is prepared by the Brooklyn Varnish Company under the trade name of Tufon #74.

The vinyl resins tested as sealing agents showed great variation in physical properties and resistance to mounting media. Their advantages are ease of solubility in a wide range of organic solvents and clear, glass-like appearance. A vinyl acetate resin (Balkelite Corporation No. AYAF) proved most useful as a sealing compound, although its texture, chemical inertness, and adhesive properties are inferior to phenolic resins. It may be dissolved to desired viscosity in 95 per cent ethyl alcohol, butyl acetate, dioxane, ethylene dichloride, and other organic solvents. The vinyl acetate polymer appears to be inert to glycerin. The solubility of certain vinyl resins in ethyl alcohol containing as much as 50 per cent water suggests its use in the preparation and embedding of whole mounts of plant and animal tissues in which complete dehydration should