24° in a glass chamber equilibrated at least 8 h previously with the solvent. The plates were then dried and the spots located by spraying with concentrated sulfuric acid containing 0.5 % potassium dichromate. The spots were identified by use of reference standards. The respective findings are presented in Table I. Generally sharp separations resulted, except for mixtures I and VIII. Of great interest is the observation that the sequence of separation of components appeared to parallel the respective boiling points of the alcohols.

This investigation was supported by Public Health Service Grant, CA 06487, from the National Cancer Institute.

Department of Biochemistry, Northwest Institute for Medical Research, Chicago, Ill. (U.S.A.)

ERIC J. SINGH
LEON L. GERSHBEIN

1 D. D. LAWSON AND H. R. GETZ, *Chem. Ind. (London)*, (1961) 1404.
2 R. SUBBARAO, M. W. ROOMI, R. R. SUBBARAM AND K. T. ACHAYA, *J. Chromatog.*, 9 (1962) 295.

Received November 23rd, 1965

J. Chromatog., 23 (1966) 180–182

Electrophoresis and detection of proteins on thin layers of alumina

The usefulness of electrophoretic techniques for separation of proteins is well-established. In recent years chromatography and electrophoresis on thin layers have been used to advantage in separations of a great variety of organic and inorganic compounds. Thin-layer techniques are frequently favored over chromatography or electrophoresis on, for example, paper because they are far faster and permit a wide variation in composition of supporting medium. Thin-layer chromatography has been used to some extent on proteins, but the only solid supports that have been used are hydroxyl-apatite[1] and various forms of Sephadex[2–7] (Pharmacia, Uppsala, Sweden), a gel filtration medium composed of cross-linked dextran. We are not aware of any published reports of electrophoresis of proteins on thin layers.

A major difficulty in the application of thin-layer techniques to proteins has been the locating of the proteins on the finished plates[6]. Conventional methods for staining proteins on paper strips[8] include steps involving washings, and are therefore inapplicable to thin-layer plates. MORRIS[6] has succeeded in circumventing this problem by overlaying the developed plates with filter paper, but a simpler method is clearly desirable. One method is outlined in this report.

Experimental

Glass plates (20 × 20 cm) were coated with alumina (aluminum oxide G, Stahl) thin layers by the usual methods. These were equilibrated with 0.1 *M* phosphate buffer (pH 7.7), and spotted with 2–10 μg of trypsin, α-chymotrypsin, or bovine

serum albumin. Electrophoresis was conducted on an RSCo. Model E-800-2B Electrophoresis Migration Chamber (Research Specialties Co., Richmond, California) at 400 V, with water cooling. The contact between the thin-layer plate and buffer trough was via filter paper wicks. Current varied from 44 to 56 mA. At the conclusion of the electrophoresis, the plates were dried, then sprayed with 2,7-dichlorofluorescein (0.2% in methanol) and viewed under ultraviolet light.

Results and discussion

Under the conditions described, trypsin, chymotrypsin and serum albumin migrated at the rate of 2.5 to 3.0 cm/h. This would suggest that electrophoresis on thin layers is potentially in the category of "rapid-electrophoresis" techniques. The migration of the applied spots was accompanied by very little if any increase in spot size, and no tailing was observed. The detection method used is comparable to that described by MORRIS[6] in sensitivity. MORRIS reports the lower limit to be about 1 μg of protein; using 2,7-dichlorofluorescein we find 2 μg of protein as a detectable amount, with 10 μg giving very obvious, well-defined spots. A possible drawback to the method described here is that prolonged exposure to the ultraviolet lamp resulted in gradual disappearance of the spots.

Although we have not conducted extensive studies, it seems reasonable to expect that the advantages of thin-layer electrophoresis, as opposed to paper, found for low molecular weight compounds apply to proteins as well.

Monsanto Research Corporation, Boston Laboratory, Everett, Mass. (U.S.A.)

DOLORES V. LOPIEKES
FRANK R. DASTOLI
STEVEN PRICE

1 A. F. HOFMANN, *Biochim. Biophys. Acta*, 60 (1962) 458.
2 B. G. JOHNSON AND L. RYMO, *Acta Chem. Scand.*, 16 (1962) 2067.
3 T. WIELAND AND H. DETERMANN, *Experientia*, 18 (1962) 431.
4 H. DETERMANN, *Experientia*, 18 (1962) 431.
5 B. G. JOHNSON AND L. RYMO, *Acta Chem. Scand.*, 18 (1964) 217.
6 C. J. O. R. MORRIS, *J. Chromatog.*, 16 (1964) 167.
7 P. ANDREWS, *Biochem. J.*, 91 (1964) 222.
8 R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1958.

Received December 9th, 1965