CHROM. 5481

A rapid micromethod for electrophoresis in Pevikon on thin-layer plates*

Pevikon C-870®, a powdered copolymer of polyvinyl chloride and polyvinyl acetate, has been used as a supporting medium for preparative zone electrophoresis[1–4]. The advantages of Pevikon for this purpose are that electro-osmosis is negligibly small, there is no interaction with serum proteins, resulting in high recovery of protein, and the material is a more suitable supporting medium for subsequent carbohydrate determinations than either granular starch or ethanolized cellulose[1]. Despite these desirable properties, previous use of Pevikon has been limited to preparative electrophoresis in blocks.

In this report, a simple and rapid method of analytical electrophoresis in a thin layer of Pevikon is described. The technique has proved useful for studies of the interaction between small molecules and proteins, such as the interaction between thyroid hormones and serum or tissue proteins.

Methods

Preparation of thin-layer plates. Pevikon C-870 was obtained from Mercer Chemical Corporation, New York, U.S.A. Fifty g of powdered Pevikon C-870 are mixed with 100 ml of dilute buffer**, and the mixture is magnetically stirred until a homogeneous suspension is obtained. The thin slurry is allowed to settle for 4 min, and 50 ml of the supernatant are then discarded. The thick slurry is mixed well with a glass rod, and is applied on an 11 × 20-cm glass plate on an aligning tray using a spreader (Desaga, Brinkmann, G.F.R.) which has been set for a thickness of 0.875 mm. The Pevikon is allowed to settle for 4 min, and then an approximately 1-cm wide strip of Pevikon is scraped off all four sides of the plate with the aid of the straight edge of another glass plate. The thickness of settled Pevikon is 0.7 mm.

Electrophoretic procedure. Electrophoresis is carried out in a Beckman Model R electrophoresis cell containing 500 ml of the electrode buffer in each chamber, placed in a horizontal position in a cold room at 10°, as shown in Fig. 1. The Pevikon thin-layer plate made is mounted on the electrophoresis cell, and a 1 × 19.5-cm Beckman wick paper strip, dampened with the electrode buffer, is placed at both anodic and cathodic ends of the Pevikon layer. Further, a 1.5 × 19.5-cm Whatman 3MM filter paper strip dampened with the same buffer is used as a bridge between the wick an Pevikon layer to insure a proper electrical connection. Whatman 3MM filter paper strips wetted with the electrode buffer are used for electrical connection between the electrode chambers and the Pevikon plate.

A 0.9-cm wide piece of microscopic cover glass inserted into a holder is used to apply samples. 10 μl of serum containing a small quantity of bromophenol blue are placed on one side of the glass, spread evenly along the edge. The glass tip, with sam-

* Supported in part by Grant No. AM-09753 from The National Institute of Arthritis and Metabolic Diseases and by Grant No. RR-76 from the Division of Health Resources and Facilities, National Institutrs of Health, Bethesda, Md., U.S.A.

** Two buffer solutions are employed. The first, or "electrode chamber buffer", is composed of tris(hydroxymethyl)aminomethane, 0.073 *M*, maleic anhydride, 0.073 *M*, and sodium hydroxide, 0.10 *M*. A second, "dilute buffer", is prepared by mixing the electrode buffer with an equal volume of distilled water. The pH of the buffers is 9.0.

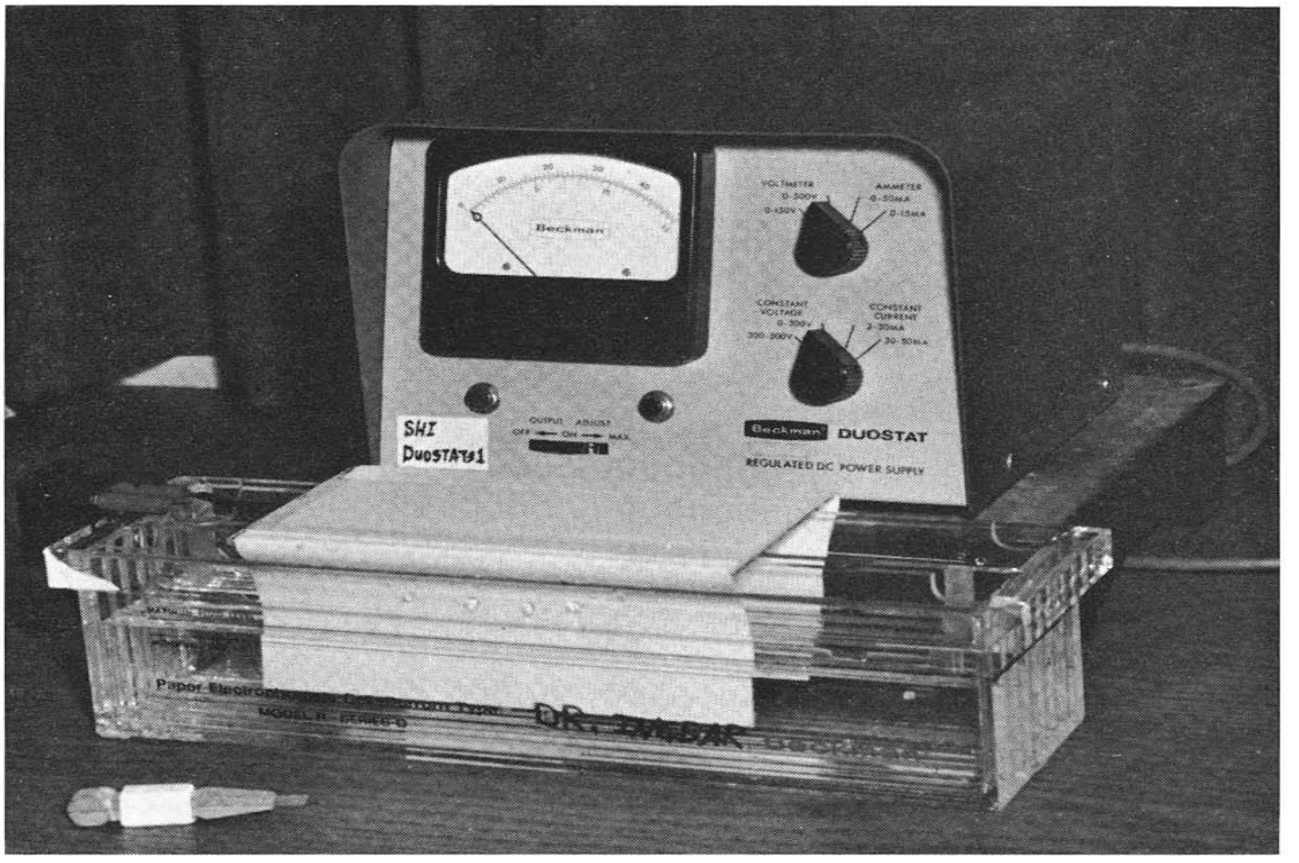

Fig. 1. Thin-layer plate in place on electrophoresis cell just prior to initiation of electrophoresis. Sample applicator is shown on a bench in front of electrophoresis cell.

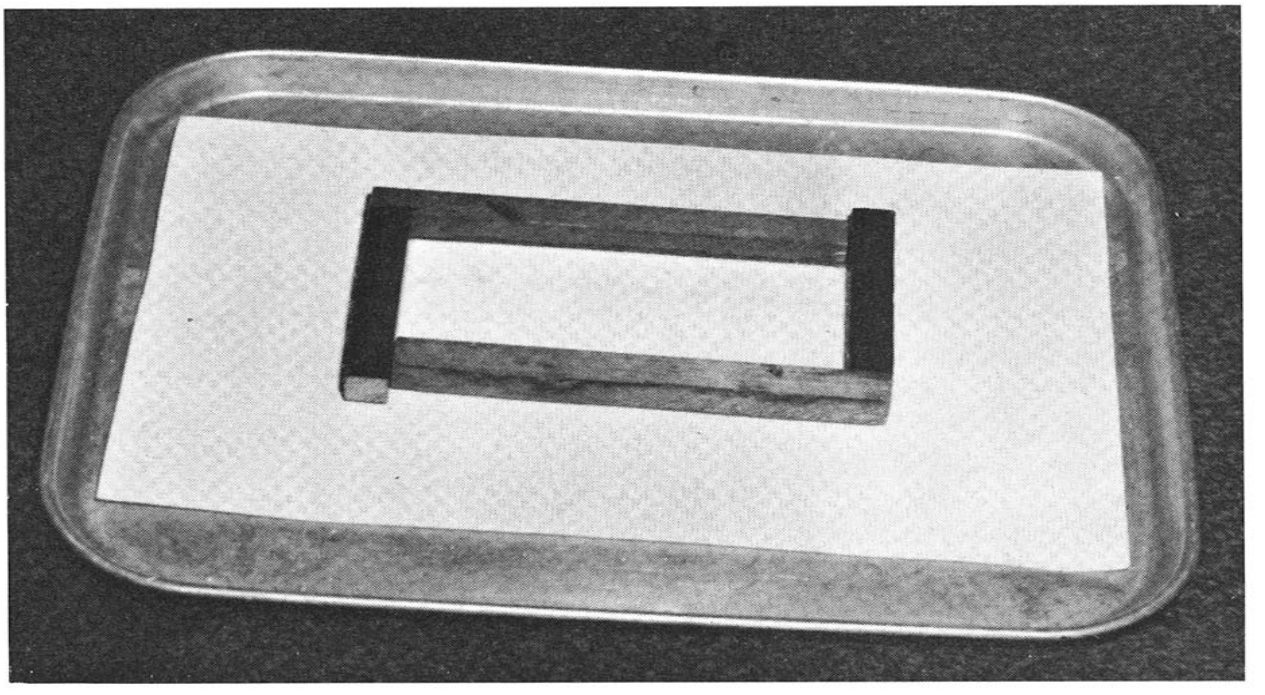

Fig. 2. Frame for drying thin-layer plate.

ple facing the anode, is pressed down vertically into the Pevikon layer 1.5 cm from its cathodic end. Eight samples can be applied at intervals of about 1 cm.

Electrophoresis is run at a constant potential of 200 V (10 V/cm) for 2 h until the albumin band stained with Bromophenol Blue moves approximately 4.5 cm.

Drying of the plate. During the drying procedure, water tends to evaporate more rapidly from the edges of the Pevikon layer than from its center, distorting the electrophoretic patterns. Therefore, drying is carried out in a horizontal position within a specially constructed frame composed of two L-shaped pieces of wood of an appropriate size so that, when these are brought together to form a rectangle (Fig. 2), the inner edges of the frame are in contact with and occlude the four sides of the Pevikon layer. The plate is then heated at 150° for 10 min until the color of the Pevikon becomes slightly pink.

Autoradiography. If autoradiographs of the electrophoretic patterns are desired, the dried Pevikon plate is wrapped tightly with Resinite® (Bordon Chemical, North Andover, Mass., U.S.A.) and is applied directly to no-screen *X*-ray film.

Quantitation of radioactivity in discrete zones. Radioactive zones are localized by means of the corresponding autoradiograph. The plate is then uniformly moistened by a fine spray of distilled water delivered from a Chromatosprayer® (Scientific Manufacturing Industries, Emeryville, Calif., U.S.A.). A surgical blade is used to incise the Pevikon layer to isolated the discrete radioactive zones. These are then scraped off the plate with a small spatula and transferred into counting tubes. Small fragments of Pevikon remaining on the plate and spatula are carefully wiped up with wet cotton-tip applicators, and are added to the counting tube together with the applicators. A similar procedure can be used to remove any zone on the plate for any analysis desired.

Staining of the plate. Dried plates are stained with 1% Amido Black 10B in 5% trichloroacetic acid in a rectangular flat-bottomed container. The plates are gently submerged beneath the staining solution to avoid occlusion of air bubbles. Proteins are stained in 5–10 min. The staining solution may be re-used after filtration.

Excess dye remaining in the plate is washed away with 5% acetic acid by the following procedure. The plate is held in a tilted position and is covered with Whatman 3MM filter paper dampened with the wash solution. A reservoir containing 5% acetic acid solution is placed at a higher level than the plate, supplying the solution continuously to the plate through a filter paper bridge. For more rapid washing, excess wash solution may be poured with a pipette on to the filter paper covering the plate. The filter paper containing absorbed dye may be changed to expedite the washing process.

After washing, the Pevikon plate is dried at room temperature overnight, and Protective Lacquer Spray® (Sigma Chemical Co., St. Louis, Mo., U.S.A.) is applied to preserve the electrophoretograms and to make the stained bands more clearly visible.

Results

Fig. 3 shows a typical electrophoretic pattern of human serum enriched with trace concentrations of ^{131}I-labeled thyroxine (T_4) or triiodothyronine (T_3). Also shown are autoradiographs of the plate, which also contained labeled T_4 and T_3 without proteins. As in moving boundary and other systems of zone electrophoresis,

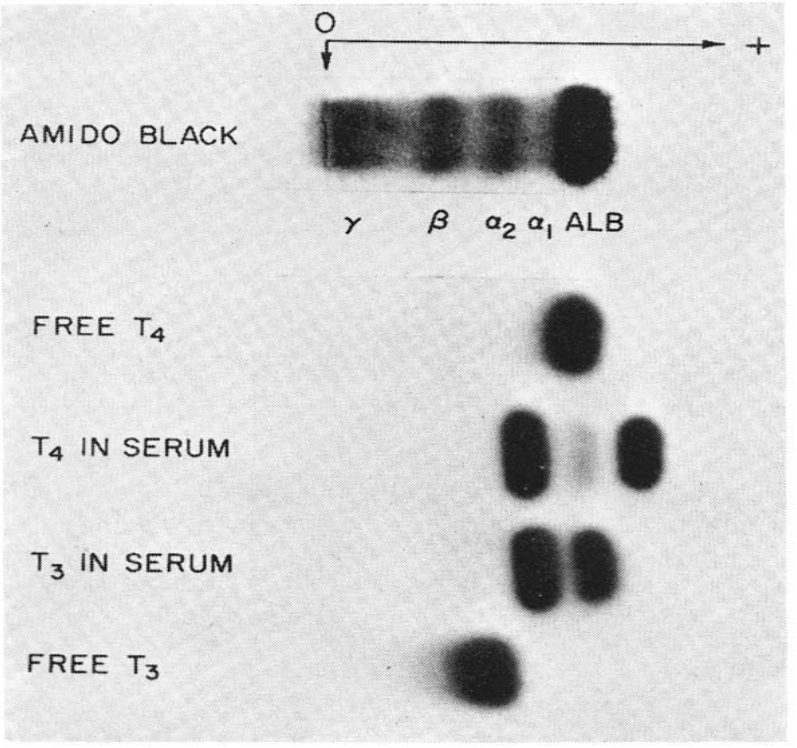

Fig. 3. Thin-layer Pevikon electrophoresis of ^{131}I-labeled thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3), either free or in normal human serum. Localization of protein bands shown above by Amido Black stain. Localization of labeled hormones shown in autoradiographs below.

serum proteins were separated into five components. In contrast with other systems of zone electrophoresis, however, gamma-globulin moved toward the anode because of the absence of electro-osmosis.

^{131}I-T_4 in serum was found in three bands, corresponding to T_4-binding prealbumin (TBPA), albumin and T_4-binding alpha-globulin (TBG), while ^{131}I-T_3 in serum was found in only two bands, corresponding to TBG and albumin. These findings were consistent with results obtained in other supporting media. It is noteworthy, however, that no significant amount of ^{131}I-T_3 remained in beta- and gamma-globulin areas, whereas, in most other systems, considerable trailing of T_3 into these areas is found because of the interaction between T_3 and the supporting medium.

T_4 in the absence of protein ("free T_4") migrated in a rather diffuse band, slightly more slowly than did serum albumin; "free T_3" migrated even more slowly, at approximately the same rate as the beta-globulin. Trailing of the free hormones was not observed.

Other experiments were performed to evaluate the affinity of Pevikon for T_4 and T_3. Tracer concentrations of the ^{131}I-labeled hormones were added to either the dilute buffer, whole rat serum or the soluble supernatant of a 33% homogenate of rat liver in 0.25 *M* sucrose. 1 ml of Pevikon slurry (1 g/ml of dilute buffer) was added to 50 μl of the preceding solutions in test-tubes. After thorough mixing, the tubes were centrifuged, the supernatants decanted, and the Pevikon was washed twice with 2 ml of dilute buffer. Table I lists the proportion of radioactivity remaining with the Pevikon in each of the three solutions and clearly demonstrates the low affinity of Pevikon for T_4 and T_3.

TABLE I

ADSORPTION OF ^{131}I-T_4 AND ^{131}I-T_3 ON PEVIKON IN THE PRESENCE AND ABSENCE OF PROTEINS
The values are the mean of duplicate determinations.

	Before wash (%)	*% Remaining with Pevikon*			
		After 1st wash		*After 2nd wash*	
		T_4	T_3	T_4	T_3
Buffer solution, pH 8.6	100	13.9	21.2	3.3	8.7
Rat serum	100	13.1	19.7	2.3	6.8
Rat liver cytosol	100	15.0	19.0	3.0	5.8

Table II presents values for the recovery of ^{131}I-labeled T_4 and T_3 from thin-layer plates following electrophoresis. Whether applied free, in rat serum, or in liver cytosol, more than 95% of T_4 and T_3 was recovered.

Discussion

Electrophoresis in Pevikon blocks has been used as a preparative procedure because of minimal electro-osmosis and minimal interaction with proteins. Moreover, because it is a non-swelling inert medium that contains about 50% of free water, the separations obtained resemble those found with free electrophoresis. However, Pevikon blocks are cumbersome and time-consuming and also require large amounts of sample if they are to be employed for analytical purposes. The present report de-

TABLE II

RECOVERY OF ^{131}I-T_4 AND ^{131}I-T_3 FROM PEVIKON PLATES AFTER ELECTROPHORESIS

	Recovery (%)
T_4	96.9
T_3	99.8
T_4 in rat serum	104.1
T_4 in rat liver cytosol	101.1
T_3 in rat liver cytosol	97.3

scribes a micromethod which is suitable for analytical purposes and which takes advantage of the favorable properties of Pevikon. Electrophoresis is performed on thin-layer plates in which Pevikon acts as the supporting medium. The method is simple and time-saving, since good separation of serum proteins can be obtained during a 2-h period of electrophoresis. Moreover, autoradiographs can be readily performed and proteins stained on the plate with little distortion due to shrinkage. Finally, the method is especially useful in studying protein-binding interactions, owing to the very low affinity of Pevikon for protein and other ligands.

In view of the current interests of this laboratory, the method was developed as a means of studying the thyroid hormone-binding proteins of tissues. In our hands, previous methods of analytic electrophoresis, *i.e.* electrophoresis in filter paper, disc gel or agarose, were found to be unsatisfactory for this purpose because of dissociation

of hormone, binding of hormone to supporting media, or poor resolution of the proteins. With the present method, it has been possible to demonstrate distinct binding proteins for T_4 and T_3 in rat liver cytosol, as will be reported elsewhere. With respect to studies of the binding of thyroid hormones in serum, particularly the binding of T_3, it also appears preferable to other procedures since it neither favors dissociation of the hormone–protein bond, as is the case in disc gel electrophoresis, nor suffers from interaction of the hormone with the supporting medium, as is the case with filter paper.

The authors wish to thank Mr. MARC BROWNING for his excellent technical assistance.

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Hospital, and The Department of Medicine,
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Received June 1st, 1971