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Thin-layer electrophoresis of indole derivatives

The chromatography of indole derivatives has been a subject of much interest for some time. A number of articles dealing with the separation of indoles of plant origin and animal origin have appeared in the last few years. Thin-layer chromatography (TLC) especially has proved to be a very useful technique[1-4]. The most recent development has been the use of "gradient TLC" as proposed by STAHL AND DUMONT[5]. Thin-layer electrophoresis (TLE) on the other hand has not been widely applied. However, that TLE can be of great value is well documented by some recent reports on the separation of polynuclear aza heterocyclic compounds[6] and in the fractionation of plant extracts[7]. Also STAHL[8] has added an entirely new chapter on TLE in his recent book on TLC, which suggests that TLE can be of value in the separation of different categories of compounds. In view of lack of knowledge on the use of TLE for the separation of indoles, it was thought to be worthwhile to study the behaviour of some physiologically important indole derivatives by means of electrophoresis and a preliminary survey of commercially available indoles using TLE was made.

Materials

Apparatus. A Shandon electrophoresis chamber was used for TLE separations in this study. Thin-layer plates were also prepared using a Shandon applicator and TLC kit.

Standards. Indole-3-acetic acid (IAA), DL-3-indole lactic acid (ILA), indole acrylic acid (IAcA), indole acetonitrile (IAN), L-tryptophane (TP), DL-5-hydroxytryptophane (5-HTP), DL-5-fluorotryptophane (5-FTP), tryptamine hydrochloride (T), and 5-hydroxytryptamine creatinine sulphate (5-HT) were purchased from Nutritional Biochemicals Corporation. The chemicals 3-indole glyoxylic acid (IGA) and 5-hydroxyindole-3-acetic acid (5-HIAA) were purchased from Sigma Chemicals Company: *v*-(indole-3)-*n*-butyric acid (IBA) was obtained from Eastman Kodak Company. All other chemicals used were purchased from Fischer Scientific Company and used without further purification.

Buffers. Borate, pH 10.0: A quantity of 7.5 g of boric acid and 3.75 g sodium hydroxide were dissolved in distilled water and made into a solution of 2.5 l in volume.

Borate, pH 12.4: An amount of 3.9 g boric acid and 4 g sodium hydroxide were dissolved in distilled water and made 1 l in volume.

Acetate: Sodium acetate solution (0.1 *N*) was adjusted to the desired pH by adding a normal sodium hydroxide solution.

Formic acid–glacial acetic acid, pH 2.0: Amounts of 78 ml of formic acid (85–90 %) and 148 ml of glacial acetic acid were mixed and diluted with distilled water to make 2.5 l.

Pyridine–glacial acetic acid, pH 6.1: Pyridine (100 ml) and 5 ml of glacial acetic acid were mixed and diluted with distilled water to make 2.5 l.

Methods

Thin-layer plates of 0.5 mm thickness were prepared (STAHL[8]). Silica Gel G (Merck) was used as the sorbent. The plates were heated at 100° for 30 min before

use. The standard indole compounds were dissolved in ethanol or water to give a concentration (of the compound) of 1 mg/ml. A quantity of 2–5 μg of each indole was spotted on to the plate for electrophoresis or chromatography. The TLC was performed in conventional glass tanks. The indoles were located by spraying the plates with a 1 % solution of *p*-dimethylaminocinnamaldehyde in concentrated HCl (w/v). The reagent was always prepared just before use and diluted with ethanol in 1:4 proportion (v/v) before spraying.

For TLE the 20 × 20 cm plate was spotted with the standard indole compounds and the plate was sprayed very gently with the buffer solution. It is extremely important that excess of buffer on the plate be avoided because it may result in the displacement of the original spot samples besides destroying the uniformity of the gel layer. The cathode (−) and anode (+) edges of the plate were connected with the buffer solution in the electrophoresis chamber by means of paper strips wetted with buffer. To avoid condensation, cold water was run in the jacket around the electrophoresis chamber. This was absolutely essential if the separation time was over 60 min and the voltage in use was high. Cold water flow also keeps the temperature of the buffer from rising very high which could otherwise result in the concentration of the buffer solution and thus a change in pH. Immediately after the required time, plates were taken out of the chamber and dried in a fumehood. If TLC were to be performed, the dried plate was put in a glass tank having an appropriate solvent. Otherwise the plate was immediately sprayed with the chromogenic reagent, dried, and then heated at 100° for 5–10 min to develop the spots; heating was not always necessary.

Results and discussion

The electrophoretic mobilities of the test substances under various combinations of current strength and buffer solutions are reported in Tables I and II. Electrophoretic mobility is expressed in arbitrary units 10^{-6} $g^{\frac{1}{2}}$ $cm^{-1\frac{1}{2}}$ by calculating from the following formula[9]:

$$U = dl/te$$

where

U is the mobility in 10^{-6} $g^{\frac{1}{2}}$ $cm^{-1\frac{1}{2}}$
l is the length of electrophorogram in cm
d is the distance travelled by the test substance in cm
t is the time in h
e is the current in V.

The movement of the compounds towards the cathode or the anode is expressed by the use of a + or − sign before the figures for electrophoretic mobility in Tables I and II.

The movement of neutral and basic indoles was very rapid in all the buffer solutions used and under all the voltages applied. However, a high voltage (500 V) acting over a small time (1 h) gave the best results. Both T and 5-HT moved very rapidly; at lower pH T moved faster than 5-HT. This behaviour was reversed when borate buffer of pH 10.0 or 12.4 was used. Similarly IAN showed a faster migration rate at higher pH and high voltage. The best separation of TP and 5-HTP took place only with borate buffer (pH 12.4) at 500 V (Fig. 1). For the separation of a mixture of nine common and important indole derivatives a system employing acetate buffer

TABLE I

ELECTROPHORETIC MOBILITES OF INDOLES AT DIFFERENT VOLTAGES[a]

A: acetate buffer, pH 5.2; current, 110 V; time, 5 h. B: pyridine–acetic acid, pH 6.1; current, 440 V; time, 1.5 h. C: acetate buffer, pH 6.0; current, 220 V; time, 3 h. D: acetate buffer, pH 6.2; current, 220 V; time, 3 h. E: pyridine–acetic acid, pH 6.1; current, 220 V; time, 3 h. F: borate buffer, pH 10.0; current, 220 V; time, 3 h.

Indoles	*Electrophoretic systems*					
	A	*B*	*C*	*D*	*E*	*F*
IAA	+0.10	+0.10	+0.07	+0.07	+0.15	+0.08
IAN	−0.05	−0.04	−0.05	−0.05	−0.03	−0.12
IBA	+0.06	+0.05	+0.03	+0.04	+0.12	+0.04
TP	−0.05	−0.01	−0.07	−0.07	−0.05	−0.11
T	−0.24	−0.20	−0.20	−0.23	−0.19	−0.21
5-HT	−0.29	−0.17	−0.23	−0.25	−0.20	−0.24
5-HTP		−0.02	−0.08	−0.07	−0.07	−0.12

[a] The electrophoretic mobility is expressed in arbitrary units; for details see text.

TABLE II

ELECTROPHORETIC MOBILITY OF INDOLES AT 500 V[a]

A: formic acid-acetic acid, pH 2.0. B: pyridine-acetic acid, pH 6.1. C: borate, pH 10.0. D: borate, pH 12.4. Time, 1 h.

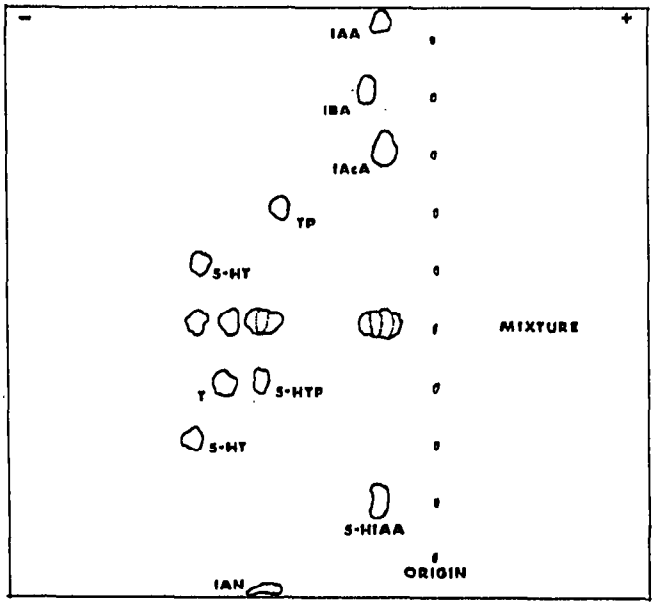
Indoles	*Buffer systems*			
	A	*B*	*C*	*D*
IAA	−0.04	+0.11	+0.04	−0.06
IAN	−0.05	−0.10	−0.16	−0.19
IBA	−0.06	+0.09	+0.02	−0.08
IAcA	−0.04	+0.08	+0.03	−0.06
5-HIAA	−0.06	+0.10	+0.01	−0.07
ILA		+0.06		
IGA		+0.12		
TP	−0.13	−0.09	−0.15	−0.18
5-FTP		−0.06		
5-HTP	−0.12	−0.08	−0.16	−0.20
T	−0.24	−0.25	−0.22	−0.24
5-HT	−0.19	−0.28	−0.28	−0.28

[a] Electrophoretic mobility is expressed in arbitrary units; for details see text.

(pH 6.0) was found to be the most suitable (Fig. 2). The time required for separation was 3 h because the movement of IAA and IBA was very slow.

The separation of indolic acids was not very clear in any of the buffer solutions and current strength described for basic or neutral compounds. The electrophoretic mobility did not give sufficient data to warrant identification, except for the colour of the spots produced by the chromogenic reagent. Only pyridine–acetic acid buffer (pH 6.1) was found useful for the separation of IAA, IBA, IAcA and 5-HIAA at a voltage of 500 V. Both IAA and IBA could also be separated easily by the use of acetate buffer (pH 6.0) and a 220 V current. Borate buffer of any strength was not useful for separating acidic indole compounds.

During the later stages of this work, a combination of the electrophoretic and the chromatographic technique proved to be the best procedure for separating acidic



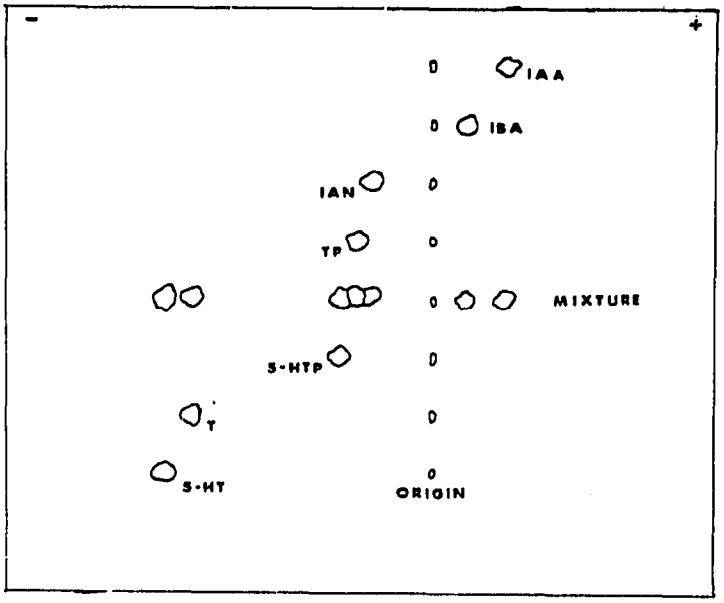


Fig. 1. Thin-layer electropherogram of indole derivatives in borate buffer, pH 12.4. Time, 1 h.

Fig. 2. Thin-layer electropherogram of indole derivatives in acetate buffer, pH 6.0. Time required to separate the components of the mixture was 3 h.

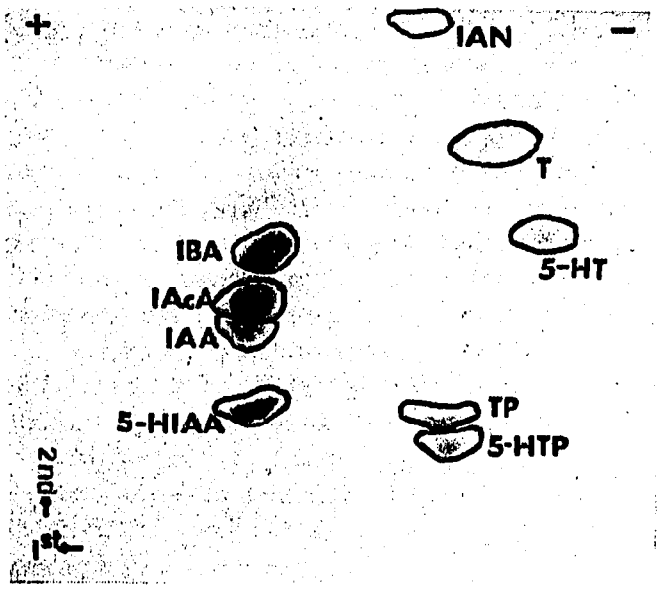


Fig. 3. A mixture of nine indoles was subjected to electrophoresis in the 1st direction using borate buffer pH 12.4; time, 1 h. Chromatography was performed in the 2nd direction using isopranol–ammonia–water (8:1:1) as the solvent system.

indoles. A mixture of nine indoles was subject to electrophoresis using borate buffer (pH 12.4) and a current of 500 V. After drying the plate, it was chromatographed in a second direction using isopropanol–ammonia–water (8:1:1, v/v) as the solvent (Fig. 3). The separation of all the components of the mixture was quite good and easily reproducible. Therefore, for acidic indoles a combined electrophoresis and chromato-

graphic technique is much more useful, while for basic and neutral indoles either technique could be used without much problem.

It is hoped that this TLE procedure may be of help to researchers in the field of biogenic amines (especially 5-HT) besides being of some value for those working on the metabolism of TP or IAA, where often it is necessary to separate a mixture of indolic substances. It would be advisable to extend the TLE procedure to a study of more compounds; moreover, a study of the use of different adsorbents could yield valuable results.

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