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THIN-LAYER ELECTROPHORESIS OF ALKALOIDS

ALFRED S. C. WAN
School of Pharmacy, University of Singapore, Sepoy Lines, Singapore 3 (Singapore)

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SUMMARY

A procedure for the thin-layer electrophoresis of alkaloids on glass plates coated with cellulose powder is described. Electrophoresis is carried out in acid and alkaline electrolytes at 500 V and 3000 V. The relative mobilities of the alkaloids are given. The easy separation of codeine from pharmaceutical preparations is demonstrated.

INTRODUCTION

The paper electrophoresis of alkaloids has been extensively studied by PARIS and co-workers[1-4], MARINI-BETTÒLO AND COCH FRUGONI[5] and INTRONA[6]. Generally low voltages were used and the electrophoresis required 2–3 h. High voltages have been used by PARIS and co-workers[2,4], PHILLIPSON AND MELVILLE[7] and WERNER[8]. The electrophoretic runs ranged from 30 to 180 min. However, the use of paper electrophoresis for the separation of alkaloids does not seem to have become widespread, possibly due to the problems associated with the lengthy electrophoretic run required, heat generation, electroendosmosis and the difficulties of handling wet paper.

A technique of thin-layer electrophoresis (TLE), developed by HONEGGER[9] and PATUSKA AND TRINKS[10], has been applied to the separation of phenols[9], periodate and iodate[11], amines and amino acids[9], food colours[12] and glycosides[13]. The separations obtained with TLE are said to be superior to those obtained by TLC and the spots are sharper and more compact. The method combines the ease of handling and rapidity of TLC with the specificity of electrophoresis. It would thus appear to be well suited for the separation and identification of alkaloids.

This paper describes in detail the technique used for the TLE of some pharmaceutically important alkaloids. The relative electrophoretic mobilities of the alkaloids are given.

EXPERIMENTAL

Apparatus and materials

A Baird and Tatlock constant voltage/constant current electrophoresis apparatus, 0–500 V.

A Camag high voltage electrophoresis apparatus 0–5000 V.
A Desaga TLC applicator.
Cellulose MN 300 (Macherey, Nagel & Co.).
Alkaloids, B.P. quality.
Solvents, analytical grade.
Codeine phosphate tablets, B.P.; compound codeine tablets, B.P.; codeine linctus, B.P.C. and codeine phosphate syrup, B.P.C.

Preparation of the plates

15 g cellulose MN 300 were mixed with 90 ml distilled water at high speed in a Virtis 45 homogeniser for 5 min. Air bubbles were removed from the slurry by evacuation with a pump. The slurry was spread by means of the Desaga applicator on 15 × 20 cm glass plates to give layers 0.50 mm thick. The plates were allowed to dry at room temperature overnight. The thin layers thus obtained were sufficiently coherent to withstand the various subsequent procedures employed.

Electrolytes

Volatile electrolytes were chosen as these could be easily and completely removed from the plates after an electrophoretic run, and thus caused no interference with the detection reagents used. The following electrolytes were found to be most suitable:

(1) Formic acid–glacial acetic acid–water (26:120:1000)
(2) Ammonia solution (0.91 sp.gr.)–water (2:98)
(3) Ammonia solution (0.91 sp.gr.)–water (10:90).

Electrophoresis

Ethanolic solutions of the various alkaloids containing 5 μg/μl were spotted 1.5 cm apart on a line 3 cm from one end of the plate. For alkaline electrolytes, the alkaloids were also spotted on a line equidistant from both ends of the plate. The plate was then saturated with electrolyte by the method adapted by McEvoy-Bowe[14] from the paper electrophoresis technique. Two pieces of Whatman No. 3MM paper were thoroughly soaked in the electrolyte and placed on a glass plate 2 cm apart. Excess electrolyte was removed by blotting until the surface of the paper appeared just shiny. The thin-layer plate was then placed, cellulose layer down, on the wet paper so that the starting line was situated half-way between the two pieces. The plate was pressed lightly to ensure good contact and in a few minutes the cellulose layer became saturated with electrolyte which migrated from both pieces of paper across the gap towards the starting line. With a little practice it was possible to make the two advancing fronts meet at the starting line.

This technique obviated the need to spot on a wet surface with the risk of damaging the layer or to spray the dry layer evenly after spotting whilst avoiding the region of the starting line. In addition, the alkaloids can be spotted either as organic or aqueous solutions. Because the electrolyte advances towards the starting line from opposite directions, a narrow compact zone of alkaloid is obtained at the origin.

Low-voltage electrophoresis

When evenly saturated, the plate was gently lifted from the paper and placed

layer down on the ribbed glass bridge provided with the BTL horizontal electrophoresis tank. Electrophoresis cellulose acetate strips (Oxoid) previously soaked in electrolyte were used as wicks. The cellulose acetate provided adequate conductivity whilst restricting the flow of fluid. Electrophoresis was carried out at a constant 500 V (33 V/cm) for 45 min in acid and for 60 min in alkaline electrolytes.

High-voltage electrophoresis

The saturated plate was placed cellulose layer down in the Camag HVE cell, mid-way between the buffer troughs. Connection to the wicks was made by means of Whatman No. 1 paper soaked in electrolyte and then blotted with filter paper. Electrophoresis was carried out at a constant 3000 V (150 V/cm) for 10 min in acid and 20 min in alkaline electrolytes.

At the end of the electrophoretic run, the plate was removed from the tank or cell and allowed to dry at room temperature in a current of air until the odour of the solvent had disappeared and the sorbent layer appeared white.

Detection of the alkaloids

Of the various modified Dragendorff reagents used for alkaloid detection, the most suitable for the present investigation was found to be VAGUJFALVI's variation[15] based on a modification of THIES AND REUTHER[16]. The reagent is an ethyl acetate solution of sodium bismuth iodide and is volatile. The reagent was diluted 1:2 with ethyl acetate for spraying. The alkaloids showed up as reddish to orange-yellow spots on evaporation of the reagent.

Colchicine and ephedrine only give a weak reaction with the Dragendorff reagent. However they can be located on the plate by exposure to iodine vapour.

Separation of codeine from pharmaceutical preparations

Codeine phosphate tablets B.P. and compound codeine tablets B.P. were powdered and shaken with sufficient water to give a concentration of 1 μg/μl of codeine phosphate and filtered. Codeine phosphate syrup B.P.C. and codeine linctus B.P.C. were appropriately diluted with water so that they contained the same concentration of codeine phosphate. The aqueous solutions were spotted on the thin-layer cellulose plates and electrophoresis carried out in acid electrolyte as described above.

RESULTS AND DISCUSSION

Table I gives the electrophoretic mobilities of nineteen pharmaceutically important alkaloids on electrophoresis at 500 V. The spots were discrete and sharp except for reserpine, which gave a very elongated spot. In acid electrolyte, quinine and quinidine were the fastest migrating alkaloids (approx. 130 mm after 45 min). The distances travelled by the alkaloids tended to vary slightly but the M_Q values (mobility relative to quinine) were found to be very constant. Satisfactory separation of the following alkaloids was obtained: atropine, brucine, cocaine, colchicine, ephedrine, ergometrine, papaverine, pilocarpine, quinine, reserpine, strychnine. However, very similar M_Q values were obtained for atropine, hyoscine, homatropine, codeine, morphine, emetine, physostigmine, tubocurarine, at an acid pH, but it was possible

TABLE I

ELECTROPHORETIC MOBILITIES OF SOME ALKALOIDS AT 500 V

15 cm × 20 cm plates coated with Cellulose MN 300, 0.50 mm thickness.

Alkaloid	*Electrolyte*				
	Formic acid–acetic acid–water (26:120:1000)	*Ammonia soln.–water (2:98)*		*Ammonia soln.–water (10:90)*	
	M_Q[a]	M_A[b]	M_A[c]	M_A[b]	M_A[c]
Atropine	0.77	1.00	1.00	1.00	1.00
Brucine	0.66	0.53	0.43	0.52	0.50
Cocaine	0.62	1.02	0.74	1.04	0.91
Codeine	0.76	0.88	0.72	0.70	0.89
Colchicine	0.41	0.82	0.75	0.85	0.75
Emetine	0.82	0.13	0.11	0.13	0.14
Ephedrine	0.87	1.22	1.15	1.16	1.13
Ergometrine	0.69	0.49	0.39	0.49	0.49
Homatropine	0.79	1.10	1.07	1.07	1.07
Hyoscine	0.77	0.98	0.76	0.96	0.90
Morphine	0.76	0.74	0.44	0.60	−0.46
Papaverine	0.67	0.19	0.15	0.22	0.20
Pilocarpine	0.88	1.00	0.78	1.02	0.87
Physostigmine	0.78	0.93	0.74	0.96	0.89
Quinine	1.00	0.28	0.25	0.31	0.34
Quinidine	1.00	0.30	0.26	0.29	0.30
Reserpine	0.47	0.08	0.07	0.09	0.11
Strychnine	0.72	0.56	0.46	0.56	0.52
Tubocurarine	0.80	0.51	0.36	0.63	0.33

[a] Mobility relative to quinine. Electrophoretic run: 45 min.
[b] Mobility relative to atropine. Electrophoretic run: 60 min. Alkaloids spotted 3 cm from end of plate.
[c] As for [b] but alkaloids spotted mid-way between ends of plate.

to separate these alkaloids by carrying out the TLE at alkaline pH. The rate of migration is slower than in acid and it was necessary to increase the electrophoretic run to 1 h. Quinine migrated slowly at alkaline pH whilst ephedrine, homatropine and atropine travelled the furthest (approx. 70 mm in 1 h). For alkaline electrolytes mobilities relative to atropine (M_A) were calculated. Migration of the alkaloids was faster in the more dilute ammonia solution due to the lower ionic strength. Examination of Table I shows that the alkaloids with similar M_Q values can be effectively separated at alkaline pH. It was also found that the location of the starting line was an important factor in effecting separation of some alkaloids. Atropine and hyoscine had similar M_A values when spotted near the end of the plate. When spotted in the mid-way position, good separation of the two alkaloids was obtained. Separation of codeine and morphine was also better when spotted in the mid-way position. These variations were most likely due to the effect of electroendosmosis. Morphine because of its phenolic OH possesses a negative charge and in 10% ammonia in the mid-way position it migrates towards the anode in contrast to the other alkaloids. Emetine was very slow moving in ammonia and physostigmine and tubocurarine had markedly different M_A values.

With high-voltage electrophoresis the migration patterns of the alkaloids were

TABLE II

ELECTROPHORETIC MOBILITIES OF SOME ALKALOIDS AT 3000 V
15 cm × 20 cm plates coated with Cellulose MN 300, 0.5 mm thickness.

Alkaloid	*Electrolyte*				
	Formic acid–acetic acid–water (26:120:1000)	*Ammonia soln.–water (2:98)*		*Ammonia soln.–water (10:90)*	
	M_Q[a]	M_A[b]	M_A[c]	M_A[b]	M_A[c]
Atropine	0.68	1.00	1.00	1.00	1.00
Brucine	0.50	0.41	0.42	0.42	0.40
Cocaine	0.42	0.89	0.90	0.83	0.83
Codeine	0.66	0.78	0.81	0.77	0.76
Colchicine	0.12	0.74	0.74	0.68	0.83
Emetine	0.73	0.09	0.11	0.10	0.11
Ephedrine	0.78	1.18	1.16	1.09	1.13
Ergometrine	0.41	0.39	0.40	0.37	0.35
Homatropine	0.71	1.07	1.10	1.04	1.04
Hyoscine	0.66	0.81	0.83	0.80	0.86
Morphine	0.66	0.60	0.58	—	−0.68
Papaverine	0.51	0.14	0.17	0.16	0.17
Pilocarpine	0.82	0.85	0.84	0.81	0.85
Physostigmine	0.64	0.81	0.82	0.77	0.74
Quinine	1.00	0.23	0.24	0.23	0.23
Quinidine	0.98	0.23	0.23	0.22	0.27
Reserpine	0.27	0.02	0.00	0.03	0.00
Strychnine	0.59	0.45	0.45	0.43	0.49
Tubocurarine	0.73	0.39	0.37	0.29	0.30

[a] Mobility relative to quinine. Electrophoretic run: 10 min.
[b] Mobility relative to atropine. Electrophoretic run: 20 min. Alkaloids spotted 3 cm from end of plate.
[c] As for [b] but alkaloids spotted mid-way between ends of plate.

essentially similar to those obtained with low voltage. The M_Q and M_A values of the alkaloids are given in Table II. Brucine and strychnine showed better separation under high-voltage electrophoresis in acid pH. It was still not possible to obtain separations between the tropane alkaloids and between codeine and morphine. Clear separations of these were obtained using alkaline electrolytes. In ammonia 10% morphine behaved as an anion and migrated towards the anode, whether spotted near the end of the plate or in the mid-way position. The variations in migration noted with electrophoresis at 500 V and differential location of the starting line were not observed with high-voltage electrophoresis. There was also little difference between the M_A values for 2% and 10% ammonia. This uniformity is due to the very high voltage applied, the short period of the electrophoretic run and the minimal electroendosmosis. It was noted that when syrup of codeine was subjected to electrophoresis at 500 V, the sugar present travelled approximately 30 mm. However, at 3000 V, the sugar remained on the starting line.

The separation of codeine from the various pharmaceutical preparations was sharp and there was no interference from the non-alkaloidal ingredients. Thin-layer electrophoresis thus offers a convenient and rapid method of separation of alkaloids

from pharmaceutical preparations without the need for elaborate and lengthy extraction procedures.

REFERENCES

1 R. PARIS AND G. FAUGERAS, *Ann. Pharm. Fr.*, 13 (1955) 359.
2 R. PARIS AND G. FAUGERAS, *Ann. Pharm. Fr.*, 16 (1958) 305.
3 R. PARIS AND G. FAUGERAS, *Ann. Pharm. Fr.*, 16 (1958) 403.
4 R. PARIS, G. DILLEMAN AND G. FAUGERAS, *Ann. Pharm. Fr.*, 17 (1959) 188.
5 G. B. MARINI-BETTÒLO AND J. A. COCH FRUGONI, *Gazz. Chim. Ital.*, 86 (1956) 1324.
6 F. INTRONA, *Med. Leg. Assicurazioni*, 12 (1964) 3.
7 J. D. PHILLIPSON AND C. MELVILLE, *J. Pharm. Pharmacol.*, 12 (1960) 506.
8 G. WERNER, in E. HEFTMANN (Editor), *Chromatography*, Reinhold, New York, 1964, p. 550
9 C. G. HONEGGER, *Helv. Chim. Acta*, 44 (1961) 173.
10 G. PATUSKA AND H. TRINKS, *Chem. Ztg.*, 85 (1961) 535.
11 F. DOBICI AND G. GRASSINI, *J. Chromatogr.*, 10 (1963) 98.
12 W. J. CRIDDLE, G. J. MOODY AND J. D. R. THOMAS, *J. Chromatogr.*, 16 (1964) 350.
13 C. E. MAY AND J. M. A. BROWN, *J. Chromatogr.*, 53 (1970) 399.
14 E. McEVOY-BOWE, personal communication.
15 D. VAGUJFALVI, *Planta Med.*, 8 (1960) 34.
16 H. THIES AND W. REUTHER, *Naturwissenschaften*, 41 (1954) 230.